

REMARKS/ARGUMENTS

In view of the foregoing amendments and following remarks, favorable reconsideration of the pending claims is respectfully requested.

Status of the Claims

Claims 2 and 4-9 are pending.

Claim 9 has been amended to emphasize that the claimed invention relates to a method in which the targeted CFTRs are activated by the administration of an n-alkanol so as to cause the required accumulation /concentration of the n-alkanol in the vicinity of the CFTR.

Claims 11-12 have been added.

Prior Art Rejections

Claims 2 and 4-9 have been rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 7,150,888 to Vail III et al. with evidence provided by the Swords publication, or in the alternative, under 35 U.S.C. § 103(a) as being unpatentable over Vail in view of the Swords. Applicants respectfully traverse these rejections.

In order for a prior art reference to anticipate a claim, each and every element recited in the claim must be either explicitly or inherently disclosed in the prior art reference. Simialry, in order to establish a prima facie case of obviousness, the combination of references must also disclose or suggest each and every element in the claimed invention.

In the present rejections, it is the Examiner's contention that tea oil contains 40 ppm hexanol (evidence provided by Swords) and therefore Vail's method of treating respiratory diseases in individuals suffering from cystic fibrosis by administering tea oil vapors inherently teaches the claimed method of treating a patient suffering from cystic fibrosis with a C₆-C₁₀ linear n-alkanol.

However, Vail does not anticipate the claimed invention because Vail does not disclose or suggest the following:

generating in said patient an amount sufficient of at least one linear n-alkanol selected from the group consisting of C₆-C₁₀ in the vicinity of said epithelial cell membranes a

concentration of said n-alkanol sufficient to partially or fully open said CFTR in said epithelial cell membranes.

Significantly, the treatment described in Vail would not produce a concentration of an n-alkanol in the vicinity of the epithelial cell membranes sufficient to partially or fully open said CFTR in said epithelial cell membranes. In this regard, Applicants have provided the Declaration (attached) of Professor Pascal Barbry Ph.D., a recognized expert in the field of Cystic Fibrosis.

To begin with, Professor Barbry's Declaration states that the tea tree oil treatment described in Vail is insufficient to generate a sufficient concentration of hexanol in the vicinity of the of the epithelial cell membranes to partially or fully open said CFTR in said epithelial cell membranes. See paragraphs 29-34. In particular, inhalation of the tea tree oil as taught by Vail would not be sufficient to cause an accumulation of any hexanol present in the Tea Tree Oil¹ to a level sufficient to partially or fully open said CFTR in said epithelial cell membranes (e.g., 1 mM) in the vicinity of the CFTR in the lungs of the patient. See paragraph 34 of Professor Barbry's Declaration. Thus, the declaratory evidence provided by Professor Barbry clearly shows that Vail does not disclose or suggest a method of administering a sufficient amount of hexanol in the vicinity of the of the epithelial cell membranes to partially or fully open said CFTR in said epithelial cell membranes. As such, Vail does not teach the step of generating in said patient an amount sufficient of at least one linear n-alkanol selected from the group consisting of C₆-C₁₀ in the vicinity of said epithelial cell membranes a concentration of said n-alkanol sufficient to partially or fully open said CFTR in said epithelial cell membranes, and therefore Vail does not anticipate or render obvious the claimed invention

Second, Vail cannot be modified to deliver a sufficient amount Tea Tree Oil so as to achieve a sufficient concentration of hexanol in the vicinity of the of the epithelial cell membranes to partially or fully open said CFTR in said epithelial cell membranes. As discussed in paragraphs 22-25 of his Declaration, Professor Barbry explains that in order to achieve a concentration of hexanol in the vicinity of the epithelial cell membranes sufficient to partially or fully open said CFTR in said epithelial cell membranes, it would be necessary to administer a

toxic amount of Tea Tree Oil to the patient. Professor Barbry then concludes that it would be impossible to administer Tea Tree Oil to a patient in an amount so as to generate a concentration of n-alkanol in the vicinity of the of the epithelial cell membranes that is sufficient to partially or fully open said CFTR in said epithelial cell membranes. For this additional reason, Applicants respectfully submit that the claimed invention is not anticipated or rendered obvious by the teachings of Vail and Swords.

Finally, in paragraph 26 Professor Barbry states that if were one to dilute the Tea Tree Oil to achieve a more physiologically acceptable level, the concentration of the hexanol would be so low in the final solution that it would be impossible to achieve the required concentration of hexanol in the vicinity of the patients CTFRs so as to have any clinical effect. Thus, it can be seen that the treatment described in Vail cannot be modified to arrive at the claimed invention.

New Claims 11 and 12 are patentable over the cited art for the same reasons given above.

In view of the foregoing amendments and remarks, it is respectfully submitted that the rejections under 35 U.S.C. § 103(a) have been overcome and that the pending claims are in condition for immediate allowance.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required

¹ Based on the Examiner's calculations for the amount of hexanol in Tea Tree Oil.

Appl. No.: 10/562,085
Amdt. dated 08/13/2010
Reply to Office Action of 04/13/2010

therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

Respectfully submitted,

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PATENT

Atty Docket: 033339/305755

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Confirmation No: 9648

Verrier, et al.

Art Unit: 1628

Serial No: 10/562,085

Examiner: Timothy P. Thomas

Filed: February 03, 2007

For: Use of n-alkanols as activators of the
CFTR channelJuly 27th, 2010**DECLARATION**

Commissioner of Patents

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Alexandria, VA 22313-1450

Sir:

Now comes Pascal BARBRY, who declares and states that:

1. I am Director of the Institut de Pharmacologie Moléculaire et Cellulaire, in Sophia Antipolis (France) since 2004.
2. I have been employed by the Centre National de la Recherche Scientifique since 1989.
3. I declare that I am experienced in the field of cystic fibrosis as it emerges from the brief curriculum vitae and the publication list herewith attached as annex 1.
4. I have been asked to review the details of the method of treatment described in US Patent Application 10/562,085 and explain how this method would be put into practice in accordance with the teaching of this Patent Application.
5. I have also been asked to review the comments of the US Patent Examiner made in two Communications dated July 21, 2009 and April 13, 2010 regarding prior art documents Vail et al, (US 7, 150, 888) and Swords et al., (1978) copies of both these documents and the examiners comments having been supplied to me.

**Background on the treatment of pulmonary diseases by the inhalation of
medicaments**

6. A number of substances can be administered directly into the lungs of a patient to have some clinical effect such as antibiotics, to fight lung infections caused by pathogenic microorganisms such as bacteria; bronchodilators, to reduce airway constriction; corticosteroids, to minimize inflammation in the airways; prostanoids, to relax blood vessels and enhance the flow of blood around the lungs and the transfer of oxygen into the circulatory system.

7. The most common method to administer these active substances into the lungs is to use a nebuliser, although other methods such as pressurised MDIs (Metered Dose Inhalers), inhalers with spacer devices and dry powder inhalers also exist and are routinely used.

8. For the present invention it would be very likely that a nebuliser would be used, due to the need to achieve a suitable concentration of the active ingredient, an n-alkanol, in the lungs of the patient.

9. A nebuliser is a breathing device that facilitates better inhalation of medicines that are meant for treating respiratory diseases. It converts a liquid medicine into a fine mist which is then inhaled by the patient.

10. The active ingredient is therefore present as a part of an inhalable solution and most normally sterile water or saline is used as the solvent to make up the remainder of the solution. The solvent is water or a saline solution so as to minimise as far as possible any physiological reaction in the patient's lungs to any component of the liquid medicine other than the active substance.

The Invention according to US 10/562,085

11. After reviewing US 10/562,085 I would summarise this Invention as a new method of relieving the symptoms of Cystic Fibrosis, based upon the findings of the inventors that the administration of an n-alkanol to a patient can cause the cystic fibrosis transmembrane conductance regulator channels (CFTR) to open.

12. It is the inactivity in a cystic fibrosis patient of their CFTRs which leads to the various pathologies which characterise this common inherited disease. In general terms and focussing upon the lungs of cystic fibrosis patients, a patient's lungs are morphologically normal at birth, but once an infection is established in the patient's lungs, a vicious cycle of inflammation, mucous hypersecretion and a failure to clear this mucous and so eliminate the bacterial infection, leads to bronchiolitis and obstruction of the airways which in turn leads to tissue destruction and bronchiectasis.

13. The inventors have shown that an optimum level of CFTR activation is achieved when the concentration of the n-alkanol, specifically octanol, in the experiments performed by the inventors, reaches 1mM in the vicinity of the CFTR.

14. One way of putting the method according to US 10/562,085 into practice would consist of administering to a patient a nebulised solution of an n-alkanol so as to generate in the vicinity of the CFTRs in the patient's lungs a concentration of the n-alkanol of 1mM.

15. I have noted that one administrable solution which the inventors of US 10/562,085 have proposed comprises between 10 and 1000 ppm of the n-alkanol. This solution would therefore comprise between 10 and 1000 pmm of the n-alkanol and a solvent such as water making up the remainder of the sterile solution.

16. The length of time necessary for the patient to inhale the nebulised solution so as to achieve the necessary concentration of the n-alkanol in the vicinity of a large enough number of the patient's CFTRs so as to have an effect, will depend upon a number of factors including the ability of the patient to respire, the presence of blockages and obstructions in the patients airway, as well as more mundane considerations such as the patient's size, weight and other non-clinical considerations.

17. The determinations of a dosage regime however would be a relatively simple affair given the vast body of work clinicians and researchers have performed on the administration of other active substances to the lungs of healthy and cystic fibrosis patients.

The method of treatment according to Vail

18. Vail concerns the administration of Tea Tree oil to the lungs of a patient suffering from one or more of maladies of the pulmonary system including cystic fibrosis.

19. Tea Tree oil is an essential oil which is isolated, unsurprisingly, from the Tea tree (*Melaleuca alternifolia*) which grows in the wild in Australia where it has also now been cultivated.

20. The use of Tea Tree oil as a complementary medicine is well known and its general antibiotic properties are also well known (*Melaleuca alternifolia* (Tea Tree) Oil: a Review of Antimicrobial and Other Medicinal Properties Clin. Microbiol. Rev., 2006; 19: 50 – 62, attached as annex 2).

21. I understand that the examiner is proposing that based upon Vail, a clinician would devise a similar treatment regime to that provided by the inventors but instead of using a solution comprising an n-alkanol, the clinician would instead use Tea Tree

oil which according to the other prior art article Swords et al., (1978) and the analysis of the examiner, contains hexanol (an n-alkanol) at a concentration of approximately 0.001 to 0.01 % v/v.

22. This treatment regime would therefore involve the nebulisation (or in some other way the generation of an aerosol) of the oil, which would then be inhaled by the patient so as to achieve a concentration of the hexanol in the patients lungs sufficient to activate the patient's CFTRs, corresponding to a concentration of 1mM.

23. If as the examiners believes Tea Tree oil comprises hexanol at a concentration of 0.001 to 0.01 % v/v, the examiners proposed treatment regime would probably necessitate the exposure of the patients lungs to the nebulised Tea Tree oil for the same period of time as in the treatment regime according to US 10/562,085 when the solution used for this method comprised between 10 and 1000 ppm of the n-alkanol. That is if the concentration of the n-alkanol was approximately the same it would be necessary to provide a similar overall volume of the nebulised Tree Tea Oil as for the n-alkanol solution according to US 10/562,085, so as to achieve the same concentration of hexanol in the vicinity of the patients CFTRs.

24. Tea Tree oil is known to be orally toxic with an LD50 in mice of 2.6 ml/kg and is also dermally toxic in mammals (please see page 59 of annex 2 for further details).

25. The examiners proposed treatment regime does not therefore seem possible given the inherent toxicity of tea tree oil.

26. If one were to dilute the Tea Tree Oil in a more physiologically acceptable solvent such as water, one would then face the problem of the concentration of hexanol being so low in the finalised solution that it would again be impossible to achieve the required concentration of hexanol in the vicinity of the patients CFTRs so as to have any clinical effect. Another problem with this approach is that according to annex 2, Tea Tree Oil is not very soluble in water.

27. In any event a prolonged exposure of patient's lungs to Tea Tree Oil does not seem to be the intention of the authors of Vail.

28. Vail describes the exposure of the lungs of a cystic fibrosis patient to the vapours of Tea Tree oil (or a mixture of this oil with Eucalyptus Oil) so as to prevent or treat opportunistic infections of the lungs, as indicated above such infections are a very serious problem experienced by cystic fibrosis patients. The constant use of antibiotics (e.g. Penicillin or Ampicillin) to treat such infections in cystic fibrosis patients can lead to severe complications such as antibiotic resistant infections. The

use of a substance with more generalised antibiotic activity like Tea Tree Oil, provides another means to treat a pulmonary infection and this appears to be the intention of the authors of Vail.

29. Vail does not specify the exact treatment regime they envision for putting their method into practice, but they do discuss two broad approaches which they have tried on themselves.

30. The first of these is discussed in column 5, lines 24 to 45 and in column 32, line 65 to column 34 line 28, of Vail and involves immediately prior to entering a public space the inhalation from a bottle of 'Eucalyptus rectified essential oil' by a process of deep inhalation via one nostril whilst the other nostril was held shut. The process then being repeated for the other nostril and a new round of inhalations being performed every 30 minutes in which the individual is at risk of coming into contact with a pathogenic microbe.

31. The authors of Vail indicate that this process when performed before entering a populated environment allowed one of them to remain free of colds, the flu and pneumonia for a period of three years, whereas before they had suffered from recurrent infections of the nose and chest. We do not believe the author has cystic fibrosis.

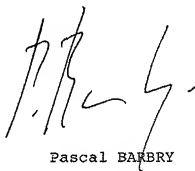
32. The second approach involves the atomisation of the Tea Tree oil/Eucalyptus oil in a piece of apparatus of their own design (figure 1 of Vail) and described at various points in Vail and particularly in column 32, line 65 to column 34 line 28. This second approach also involves, immediately prior to entering a public space, the inhalation by mouth and/or one or both nostrils the atomised Tea Tree oil from the apparatus and the subsequent readministration of the atomised oil via the selected orifice every 30 minutes that the individual remains at risk of being exposed to pathogenic microbes.

33. To summarise the two approaches described in Vail therefore, before entering a public place an individual breathes deeply from a source of Tea Tree oil (or a mixture with Eucalyptus Oil and/or another essential oil) via the mouth and/or one or both nostrils and repeats this every 30 minutes they remain in a public place.

34. Such an inhalation would not be sufficient to cause an accumulation of any hexanol present in the Tea Tree Oil to a level of 1 mM in the vicinity of the CFTR in the lungs of a patient, based upon the examiners estimated concentration of hexanol present in Tea Tree Oil according to Swords et al.

35. Vail therefore does not teach any method that would allow a clinician to put into practice the new method of treatment which the inventors of US 10/562,085 have proposed, as it does not appear to be possible to administer a sufficient quantity of Tea Tree Oil so as to achieve the required concentration of the n-alkanol in the vicinity of the patients CFTRs.

Date: July 27th, 2010

A handwritten signature in black ink, appearing to read 'P. Barbry', with a stylized flourish at the end.

Pascal BARBRY

Curriculum

- 2004 → now: Director of the Institut de Pharmacologie Moléculaire et Cellulaire, CNRS UMR6097 – France (staff = 150, 17 research groups – 10 M€ annual budget)

- 1999 → now: Group Leader, CNRS - Centre National de la Recherche Scientifique, UMR 6097, Sophia-Antipolis, France
- Creator and Head of the Nice Sophia Antipolis Functional Genomics Platform (High Throughput Sequencing, Affymetrix platform; microRNA analyses; bioinformatics, production of glass slide microarrays) and of the research team " Physiological Genomics of the Eucaryotes". 8 investigators and 7 logisticians/technicians.

- 1998–1999: Visiting Cell Biologist, UCSF- University of California San Francisco, USA
- in vivo* models to study lung ion transport. Inflammation and physiopathology of the respiratory system (Prof M.A. Matthay, UCSF).
- 1997–1998 : Research Director, 2nd class, CNRS - Centre National de la Recherche Scientifique, UPR411, Sophia-Antipolis, France
- Characterization of the first ionotropic receptor for a peptide (FMRFamide), and of new members of the ENaC-ASIC gene super-family.
- Biochemical study of the proteins belonging to the ENaC-ASIC gene super-family.
- Study of the regulation and of the expression of the amiloride-sensitive epithelial Na⁺ channel.

- 1989–1997 : Research Supervisor, CNRS -Centre National de la Recherche Scientifique, Nice - France
- Biochemistry and pharmacology of amiloride binding proteins.
- Characterization of CFTR, the cystic fibrosis gene product, and some pathological mutants (Δ F508, Δ I507, R560T, P574H, Δ Exon9).
- Pharmacology of CFTR. Characterization of NS004, the first direct CFTR activator.
- Molecular cloning of the pore forming subunits of the epithelial amiloride-sensitive Na⁺ channel.

- 2010: Member of the Scientific Advisory Board of the GIGA Cancer (University of Liège)
- 1995-1999 + since 2004: Member of the Scientific Board of the French cystic fibrosis Association
- 2000-2005 + since 2009: Member of the Advisory board at Expert Commission n°2 of ARC
- 2001-2005: National Coordinator of the Transcriptome Program, French Genopole Network
- 2001-2002: Scientific Board Member of the French Research Ministry Action "THERAPEUTICAL MOLECULES & TARGETS"
- Expert for the ANR (Programme Blanc), AERES

- 1998-99 Laureate of Perkins Memorial Award
- 1997 Award from OTAN Scientific and Technical Research Fellowship
- 1993 CNRS Bronze medal

- 1985–1990 PhD in Molecular and Cellular Pharmacology, University of Nice, France
- 1981–1984 Polytechnique School, Palaiseau, France

Main publications (111 original publications since 1984; 4331 citations; 39,02 average citations per item; h-index = 35; source = ISI)

- Potter N. et al. Identification of keratinocyte growth factor as a target of microRNA-155 in lung fibroblasts: implication in epithelial-mesenchymal interactions. *PLoS One*. 2009. 4:e6718.
- Cardinaud B. et al. miR-34b/miR-34c: a regulator of TCL1 expression in t1q- chronic lymphocytic leukaemia? *Leukemia*. 2009.
- Colell A et al. GAPDH and autophagy preserve survival after apoptotic cytochrome c release in the absence of caspase activation. *Cell* 2007; 129: 983-997
- Le Brigand K and Barbry P. Mediante : a Web-based Microarray data manager. *Bioinformatics* 2007; 23 : 1304-1306
- Triboulet R et al. Suppression of microRNA-silencing pathway by HIV-1 during virus replication. *Science* 2007 ; 315: 1579-1582
- Le Brigand K et al.. An open-access long oligonucleotide microarray resource for analysis of the human and mouse transcriptomes. *Nucleic Acids Research* 2006, 1-13.
- Fang, X et al. CFTR Dependent and Independent Mechanisms of Fluid Absorption from the Distal Air Spaces of the Mouse Lung *J Gen Physiol* 2002 119:199-207.
- Lingueglia et al. Cloning of the amiloride-sensitive FMRFamide peptide-gated sodium channel. *Nature* 1995, 378:730-733.
- Lingueglia et al. Expression cloning of an epithelial amiloride-sensitive Na⁺ channel. A new channel type with homologies to Caenorhabditis elegans degenerins. *Fabs Letters*, 1993, 318: 95-99
- Dalemans et al. Altered chloride ion channel kinetics associated with the delta F508 cystic fibrosis mutation *Nature*, 1991, 354: 526-528.

Keywords : Transcriptome, DNA microarrays, Lung, Genomics, microRNA, ion transport, pharmacology

Melaleuca alternifolia (Tea Tree) Oil: a Review of Antimicrobial and Other Medicinal Properties

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INTRODUCTION

Many complementary and alternative medicines have enjoyed increased popularity in recent decades. Efforts to validate their use have seen their putative therapeutic properties come under increasing scrutiny in vitro and, in some cases, in vivo. One such product is tea tree oil (TTO), the volatile essential oil derived mainly from the Australian native plant *Melaleuca alternifolia*. Employed largely for its antimicrobial properties, TTO is incorporated as the active ingredient in many topical formulations used to treat cutaneous infections. It is widely available over the counter in Australia, Europe, and North America and is marketed as a remedy for various ailments.

COMPOSITION AND CHEMISTRY

TTO is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes, and their associated alcohols. Terpenes are volatile, aromatic hydrocarbons and may be considered polymers of isoprene, which has the formula C_5H_8 . Early reports on the composition of TTO described 12 (65), 21 (3),

and 48 (142) components. The seminal paper by Brophy and colleagues (25) examined over 800 TTO samples by gas chromatography and gas chromatography-mass spectrometry and reported approximately 100 components and their ranges of concentrations (Table 1).

TTO has a relative density of 0.885 to 0.906 (89), is only sparingly soluble in water, and is miscible with nonpolar solvents. Some of the chemical and physical properties of TTO components are shown in Table 2.

Given the scope for batch-to-batch variation, it is fortunate that the composition of oil sold as TTO is regulated by an international standard for "Oil of *Melaleuca*—terpinen-4-ol type," which sets maxima and/or minima for 14 components of the oil (89) (Table 1). Notably, the standard does not stipulate the species of *Melaleuca* from which the TTO must be sourced. Instead, it sets out physical and chemical criteria for the desired chemotype. Six varieties, or chemotypes, of *M. alternifolia* have been described, each producing oil with a distinct chemical composition. These include a terpinen-4-ol chemotype, a terpinolene chemotype, and four 1,8-cineole chemotypes (83). The terpinen-4-ol chemotype typically contains levels of terpinen-4-ol of between 30 to 40% (83) and is the chemotype used in commercial TTO production. Despite the inherent variability of commercial TTO, no obvious differences in its bioactivity either in vitro or in vivo have been noted so far. The suggestion that oil from a particular *M. alternifolia* clone possesses en-

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TABLE 1. Composition of *M. alternifolia* (tea tree) oil

Component	Composition (%)	
	ISO 4730 range ^a	Typical composition ^b
Terpinen-4-ol	≥30 ^c	40.1
γ-Terpinene	10–28	23.0
α-Terpinene	5–13	10.4
1,8-Cineole	≤15 ^d	5.1
Terpinolene	1.5–5	3.1
p-Cymene	0.5–12	2.9
α-Pinene	1–6	2.6
α-Terpineol	1.5–8	2.4
Aromadendrene	Trace–7	1.5
δ-Cadinene	Trace–8	1.3
Limonene	0.5–4	1.0
Sabinene	Trace–3.5	0.2
Globulol	Trace–3	0.2
Viridiflorol	Trace–1.5	0.1

^a ISO 4730, International Organization for Standardization standard no. 4730 (from reference 89).

^b From reference 89.

^c No upper limit is set, although 48% has been proposed.

^d No lower limit is set.

hanced microbicidal activity has been made (106), but the evidence is not compelling.

The components specified by the international standard were selected for a variety of reasons, including provenance verification and biological activity. For example, with provenance, the inclusion of the minor components sabinene, globulol, and viridiflorol is potentially helpful, since it may render the formulation of artificial oil from individual components difficult or economically untenable. With biological activity, the antimicrobial activity of TTO is attributed mainly to terpinen-4-ol, a major component of the oil. Consequently, to optimize antimicrobial activity, a lower limit of 30% and no upper limit were set for terpinen-4-ol content. Conversely, an upper limit of 15% and no lower limit were set for 1,8-cineole, although the rationale for this may not have been entirely sound. For many years cineole was erroneously considered to be a skin

and mucous membrane irritant, fuelling efforts to minimize its level in TTO. This reputation was based on historical anecdotal evidence and uncorroborated statements (20, 55, 98, 126, 153, 156–158), and repetition of this suggestion appears to have consolidated the myth. Recent data, as discussed later in this review, do not indicate that 1,8-cineole is an irritant. Although minimization of 1,8-cineole content on the basis of reducing adverse reactions is not warranted, it remains an important consideration since 1,8-cineole levels are usually inversely proportional to the levels of terpinen-4-ol (25), one of the main antimicrobial components of TTO (36, 48, 71, 126).

The composition of TTO may change considerably during storage, with p-cymene levels increasing and α- and γ-terpinene levels declining (25). Light, heat, exposure to air, and moisture all affect oil stability, and TTO should be stored in dark, cool, dry conditions, preferably in a vessel that contains little air.

PROVENANCE AND NOMENCLATURE

The provenance of TTO is not always clear from its common name or those of its sources. It is known by a number of synonyms, including "melaleuca oil" and "ti tree oil," the latter being a Maori and Samoan common name for plants in the genus *Cordyline* (155). Even the term "melaleuca oil" is potentially ambiguous, since several chemically distinct oils are distilled from other *Melaleuca* species, such as cajuput oil (also cajeput or cajaput) from *M. cajuputi* and niaouli oil from *M. quinquenervia* (often misidentified as *M. viridiflora*) (51, 98). However, the term has been adopted by the Australian Therapeutic Goods Administration as the official name for TTO. The use of common plant names further confounds the issue. In Australia, "tea trees" are also known as "paperbark trees," and collectively these terms may refer to species in the *Melaleuca* or *Leptospermum* genera, of which there are several hundred. For instance, common names for *M. cajuputi* include "swamp tea tree" and "paperbark tea tree," while those for *M. quinquenervia* include "broad-leaved tea tree" and "broad-leaved paperbark" (98). Many *Leptospermum* species are cultivated as ornamental plants and are often mistakenly identified as the source of TTO. In addition, the essential oils kanuka and manuka, derived from the New Zealand plants *Kimzea ericoides* and *Leptospermum scoparium*, respectively, are referred to as New Zealand TTOs (42) although they are very different in composition from Australian TTO (125). In this review article, the term TTO will refer only to the oil of *M. alternifolia*.

As explained above, the international standard for TTO does not specify which *Melaleuca* species must be used to produce oil. Rather it sets out the requirements for an oil chemotype. Oils that meet the requirements of the standard have been distilled from *Melaleuca* species other than *M. alternifolia*, including *M. dissitiflora*, *M. linearifolia*, and *M. uncinata* (113). However, in practice, commercial TTO is produced from *M. alternifolia* (Maiden and Betts) Checl. The *Melaleuca* genus belongs to the Myrtaceae family and contains approximately 230 species, almost all of which are native to Australia (51). When left to grow naturally, *M. alternifolia* grows to a tree reaching heights of approximately 5 to 8 meters (45). Trees older than 3 years typically flower in October and November (12, 98), and flowers are produced in loose, white to

TABLE 2. Properties of TTO components

Component	Type of compound	Chemical formula	Solubility (ppm) ^a	Log K _{ow} ^b
Terpinen-4-ol	Monocyclic terpene alcohol	C ₁₀ H ₁₈ O	1,491	3.26
γ-Terpinene	Monocyclic terpene	C ₁₀ H ₁₆	1.0	4.36
α-Terpinene	Monocyclic terpene	C ₁₀ H ₁₆	8.2	4.25
1,8-Cineole	Monocyclic terpene alcohol	C ₁₀ H ₁₈ O	907	2.84
α-Terpinolene	Monocyclic terpene	C ₁₀ H ₁₆	4.3	4.24
p-Cymene	Monocyclic terpene	C ₁₀ H ₁₄	6.2	
(+)-α-Pinene	Dicyclic terpene	C ₁₀ H ₁₆	0.57	4.44
α-Terpineol	Monocyclic terpene alcohol	C ₁₀ H ₁₈ O	1,827	3.28
Aromadendrene	Sesquiterpene	C ₁₅ H ₂₄		
δ-Cadinene	Sesquiterpene	C ₁₅ H ₂₄		
(+)-Limonene	Monocyclic terpene	C ₁₀ H ₁₆	1.0	4.38
Sabinene	Dicyclic monoterpene	C ₁₀ H ₁₆		
Globulol	Sesquiterpene alcohol	C ₁₅ H ₂₆ O		

^a From reference 63.

^b K_{ow}, octanol-water partition coefficient, from reference 62.

creamy colored terminal spikes, which can give trees a "fluffy" appearance (155).

COMMERCIAL PRODUCTION

The commercial TTO industry was born after the medicinal properties of the oil were first reported by Penfold in the 1920s (121–124) as part of a larger survey into Australian essential oils with economic potential. During that nascent stage, TTO was produced from natural bush stands of plants, ostensibly *M. alternifolia*, that produced oil with the appropriate chemotype. The native habitat of *M. alternifolia* is low-lying, swampy, subtropical, coastal ground around the Clarence and Richmond Rivers in northeastern New South Wales and southern Queensland (142), and, unlike several other *Melaleuca* species, it does not occur naturally outside Australia. The plant material was hand cut and often distilled on the spot in makeshift, mobile, wood-fired bush stills. The industry continued in this fashion for several decades. Legend has it that the oil was considered so important for its medicinal uses that Australian soldiers were supplied oil as part of their military kits during World War II and that bush cutters were exempt from national service (35). However, no evidence to corroborate these accounts could be found (A.-M. Conde and M. Pollard [Australian War Memorial, Canberra, Australia], personal communication). Production ebbed after World War II as demand for the oil declined, presumably due to the development of effective antibiotics and the waning image of natural products. Interest in the oil was rekindled in the 1970s as part of the general renaissance of interest in natural products. Commercial plantations were established in the 1970s and 1980s, allowing the industry to mechanize and produce large quantities of a consistent product (25, 93). Today there are plantations in Western Australia, Queensland, and New South Wales, although the majority are in New South Wales around the Lismore region. Typically, plantations are established from seedlings sowed and raised in greenhouses prior to being planted out in the field at high density. The time to first harvest varies from 1 to 3 years, depending on the climate and rate of plant growth. Harvesting is by a coppicing process in which the whole plant is cut off close to ground level and chipped into smaller fragments prior to oil extraction.

Oil Extraction

TTO is produced by steam distillation of the leaves and terminal branches of *M. alternifolia*. Once condensed, the clear to pale yellow oil is separated from the aqueous distillate. The yield of oil is typically 1 to 2% of wet plant material weight. Alternative extraction methods such as the use of microwave technology have been considered, but none has been utilized on a commercial scale.

ANTIMICROBIAL ACTIVITY IN VITRO

Of all of the properties claimed for TTO, its antimicrobial activity has received the most attention. The earliest reported use of the *M. alternifolia* plant that presumably exploited this property was the traditional use by the Bundjalung Aborigines of northern New South Wales. Crushed leaves of "tea trees"

were inhaled to treat coughs and colds or were sprinkled on wounds, after which a poultice was applied (135). In addition, tea tree leaves were soaked to make an infusion to treat sore throats or skin ailments (101, 135). The oral history of Australian Aborigines also tells of healing lakes, which were lagoons into which *M. alternifolia* leaves had fallen and decayed over time (3). Use of the oil itself, as opposed to the unextracted plant material, did not become common practice until Penfold published the first reports of its antimicrobial activity in a series of papers in the 1920s and 1930s. In evaluating the antimicrobial activity of *M. alternifolia* oil and other oils, he made comparisons with the disinfectant carbolic acid or phenol, the gold standard of the day, in a test known as the Rideal-Walker (RW) coefficient. The activity of TTO was compared directly with that of phenol and rated as 11 times more active (121). The RW coefficients of several TTO components were also reported, including 3.5 for cineole and 8 for cymene (122), 13 for linalool (123), and 13.5 for terpinen-4-ol and 16 for terpineol (121). As a result, TTO was promoted as a therapeutic agent (5–7). These publications, as well as several others (60, 70, 84, 102, 120, 124, 152), describe a range of medicinal uses for TTO. However, in terms of the evidence they provide for the medicinal properties of TTO, they are of limited value, since by the standards of today the data they provide would be considered mostly anecdotal.

In contrast, contemporary data clearly show that the broad-spectrum activity of TTO includes antibacterial, antifungal, antiviral, and antiparasitic activities. Not all of the activity has been characterized well in vitro, and in the few cases where clinical work has been done, data are promising but thus far inadequate.

Evaluation of the antimicrobial activity of TTO has been impeded by its physical properties; TTO and its components are only sparingly soluble in water (Table 2), and this limits their miscibility in test media. Different strategies have been used to counteract this problem, with the addition of surfactants to broth and agar test media being used most widely (11, 13, 15, 31, 32, 61). Dispersion of TTO in liquid media usually results in a turbid suspension that makes determination of end points in susceptibility tests difficult. Occasionally dyes have been used as visual indicators of the MIC, with mixed success (31, 32, 40, 104).

Antibacterial Activity

The few reports of the antibacterial activity of TTO appearing in the literature from the 1940s to the 1980s (11, 15, 100, 153) have been reviewed elsewhere previously (35). From the early 1990s onwards, many reports describing the antimicrobial activity of TTO appeared in the scientific literature. Although there was still a degree of discrepancy between the methods used in the different studies, the MICs reported were often relatively similar. A broad range of bacteria have now been tested for their susceptibilities to TTO, and some of the published susceptibility data are summarized in Table 3. While most bacteria are susceptible to TTO at concentrations of 1.0% or less, MICs in excess of 2% have been reported for organisms such as commensal skin staphylococci and micrococci, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* (13,

TABLE 3. Susceptibility data for bacteria tested against *M. alternifolia* oil

Bacterial species	% (vol/vol)		Reference(s)
	MIC	MBC	
<i>Acinetobacter baumannii</i>	1	1	79
<i>Actinomyces viscosus</i>	0.6	>0.6	134
<i>Actinomyces</i> spp.	1	1	80
<i>Bacillus cereus</i>	0.3		61
<i>Bacteroides</i> spp.	0.06–0.5	0.06–0.12	75
<i>Corynebacterium</i> sp.	0.2–2	2	42, 61, 79
<i>Enterococcus faecalis</i>	0.5–>8	>8	13, 61
<i>E. faecium</i> (vancomycin resistant)	0.5–1	0.5–1	115
<i>Escherichia coli</i>	0.08–2	0.25–4	13, 32, 67, 104
<i>Fusobacterium nucleatum</i>	0.6–>0.6	0.25	134, 144
<i>Klebsiella pneumoniae</i>	0.25–0.3	0.25	61, 79
<i>Lactobacillus</i> spp.	1–2	2	75, 80
<i>Micrococcus luteus</i>	0.06–0.5	0.25–6	79
<i>Peptostreptococcus anaerobius</i>	0.2–0.25	0.03–>0.6	75, 134
<i>Porphyromonas endodontalis</i>	0.025–0.1	0.025–0.1	80
<i>P. gingivalis</i>	0.11–0.25	0.13–>0.6	134, 144
<i>Prevotella</i> spp.	0.03–0.25	0.03	75
<i>Prevotella intermedia</i>	0.003–0.1	0.003–0.1	80
<i>Propionibacterium acnes</i>	0.05–0.63	0.5	37, 61, 126
<i>Proteus vulgaris</i>	0.08–2	4	13, 42, 61, 104
<i>Pseudomonas aeruginosa</i>	1–8	2–>8	13, 61, 79
<i>Staphylococcus aureus</i>	0.5–1.25	1–2	13, 32, 126
<i>S. aureus</i> (methicillin resistant)	0.04–0.35	0.5	31, 42, 104, 115
<i>S. epidermidis</i>	0.45–1.5	4	42, 79, 126
<i>S. hominis</i>	0.5	4	79
<i>Streptococcus pyogenes</i>	0.12–2	0.25–4	13, 33
<i>Veillonella</i> spp.	0.016–1	0.03–1	80

79). TTO is for the most part bactericidal in nature, although it may be bacteriostatic at lower concentrations.

The activity of TTO against antibiotic-resistant bacteria has attracted considerable interest, with methicillin-resistant *Staphylococcus aureus* (MRSA) receiving the most attention thus far. Since the potential to use TTO against MRSA was first hypothesized (153), several groups have evaluated the activity of TTO against MRSA, beginning with Carson et al. (31), who examined 64 MRSA isolates from Australia and the United Kingdom, including 33 mupirocin-resistant isolates. The MICs and minimal bactericidal concentrations (MBCs) for the Australian isolates were 0.25% and 0.5%, respectively, while those for the United Kingdom isolates were 0.312% and 0.625%, respectively. Subsequent reports on the susceptibility of MRSA to TTO have similarly not shown great differences compared to antibiotic-sensitive organisms (39, 58, 68, 106, 115).

For the most part, antibacterial activity has been determined using agar or broth dilution methods. However, activity has also been demonstrated using time-kill assays (34, 48, 80, 106), suspension tests (107), and "ex vivo"-excised human skin (108). In addition, vaporized TTO can inhibit bacteria, including *Mycobacterium avium* ATCC 4676 (105), *Escherichia coli*, *Haemophilus influenzae*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae* (85). There are anecdotal reports of aerosolized TTO reducing hospital-acquired infections (L. Bowden, Abstr. Infect. Control Nurses Assoc. Annu. Infect. Control Conf., p. 23, 2001) but no scientific data.

Mechanism of antibacterial action. The mechanism of action of TTO against bacteria has now been partly elucidated. Prior to the availability of data, assumptions about its mechanism of action were made on the basis of its hydrocarbon structure and attendant lipophilicity. Since hydrocarbons partition preferentially into biological membranes and disrupt their vital functions (138), TTO and its components were also presumed to behave in this manner. This premise is further supported by data showing that TTO permeabilizes model liposomal systems (49). In previous work with hydrocarbons not found in TTO (90, 146a) and with terpenes found at low concentrations in TTO (4, 146), lysis and the loss of membrane integrity and function manifested by the leakage of ions and the inhibition of respiration were demonstrated. Treatment of *S. aureus* with TTO resulted in the leakage of potassium ions (49, 69) and 260-nm-light-absorbing materials (34) and inhibited respiration (49). Treatment with TTO also sensitized *S. aureus* cells to sodium chloride (34) and produced morphological changes apparent under electron microscopy (127). However, no significant lysis of whole cells was observed spectrophotometrically (34) or by electron microscopy (127). Furthermore, no cytoplasmic membrane damage could be detected using the lactate dehydrogenase release assay (127), and only modest uptake of propidium iodide was observed (50) after treatment with TTO.

In *E. coli*, detrimental effects on potassium homeostasis (47), glucose-dependent respiration (47), morphology (67), and ability to exclude propidium iodide (50) have been observed. A modest loss of 280-nm-light-absorbing material has also been reported (50). In contrast to the absence of whole-cell lysis seen in *S. aureus* treated with TTO, lysis occurs in *E. coli* treated with TTO (67), and this effect is exacerbated by co-treatment with EDTA (C. Carson, unpublished data). All of these effects confirm that TTO compromises the structural and functional integrity of bacterial membranes.

The loss of viability, inhibition of glucose-dependent respiration, and induction of lysis seen after TTO treatment all occur to a greater degree with organisms in the exponential rather than the stationary phase of growth (67; S. D. Cox, J. L. Markham, C. M. Mann, S. G. Wyllie, J. E. Gustafson, and J. R. Warrington, Abstr. 28th Int. Symp. Essential Oils, p. 201–213, 1997). The increased vulnerability of actively growing cells was also apparent in the greater degree of morphological changes seen in these cells by electron microscopy (S. D. Cox et al. Abstr. 28th Int. Symp. Essential Oils, p. 201–213). The differences in susceptibility of bacteria in different phases of growth suggest that targets other than the cell membrane may be involved.

When the effects of terpinen-4-ol, α -terpineol, and 1,8-cineole on *S. aureus* were examined, none was found to induce autolysis but all were found to cause the leakage of 260-nm-light-absorbing material and to render cells susceptible to sodium chloride (34). Interestingly, the greatest effects were seen with 1,8-cineole, a component often considered to have marginal antimicrobial activity. This raises the possibility that while cineole may not be one of the primary antimicrobial components, it may permeabilize bacterial membranes and facilitate the entry of other, more active components. Little work on the effects of TTO components on cell morphology has been reported. Electron microscopy of terpinen-4-ol-treated *S. aureus* cells (34) revealed lesions similar to those seen after TTO

TABLE 4. Susceptibility data for fungi tested against *M. alternifolia* oil

Fungal species	% (vol/vol)		Reference(s)
	MIC	MFC	
<i>Alternaria</i> spp.	0.016–0.12	0.06–2	74
<i>Aspergillus flavus</i>	0.31–0.7	2–4	61, 74, 116, 137
<i>A. fumigatus</i>	0.06–>2	1–2	74, 148
<i>A. niger</i>	0.016–0.4	2–8	15, 61, 74
<i>Blastosporium</i> spp.	0.25		117
<i>Candida albicans</i>	0.06–8	0.12–1	13, 42, 52, 59, 77, 111, 116, 117, 148
<i>C. glabrata</i>	0.03–8	0.12–0.5	13, 52, 59, 77, 111, 117, 148
<i>C. parapsilosis</i>	0.03–0.5	0.12–0.5	52, 77, 111, 117
<i>C. tropicalis</i>	0.12–2	0.25–0.5	52, 59, 148
<i>Cladosporium</i> spp.	0.008–0.12	0.12–4	74
<i>Cryptococcus neoformans</i>	0.015–0.06		111
<i>Epidemophyton floccosum</i>	0.008–0.7	0.12–0.25	42, 74
<i>Fusarium</i> spp.	0.008–0.25	0.25–2	74
<i>Malassezia furfur</i>	0.03–0.12	0.5–1.0	73
<i>M. sympodialis</i>	0.016–0.12	0.06–0.12	73
<i>Microsporum canis</i>	0.03–0.5	0.25–0.5	52, 74, 116
<i>M. gypseum</i>	0.016–0.25	0.25–0.5	52
<i>Penicillium</i> spp.	0.03–0.06	0.5–2	74
<i>Rhodotorula rubra</i>	0.06	0.5	71
<i>Saccharomyces cerevisiae</i>	0.25	0.5	71
<i>Trichophyton mentagrophytes</i>	0.11–0.44	0.25–0.5	52, 61, 116
<i>T. rubrum</i>	0.03–0.6	0.25–1	42, 52, 74, 116
<i>T. tonsurans</i>	0.004–0.016	0.12–0.5	74
<i>Trichosporon</i> spp.	0.12–0.22	0.12	71, 116

treatment (127), including mesosome-like structures.

Mechanism of action studies analogous to those described above have not been conducted with *P. aeruginosa*. Instead, research has concentrated on how this organism is able to tolerate higher concentrations of TTO and/or components. These studies have indicated that tolerance is associated with the outer membrane by showing that when *P. aeruginosa* cells are pretreated with the outer membrane permeabilizer polymyxin B nonapeptide or EDTA, cells become more susceptible to the bactericidal effects of TTO, terpinen-4-ol, or γ -terpinene (99, 103).

In summary, the loss of intracellular material, inability to maintain homeostasis, and inhibition of respiration after treatment with TTO and/or components are consistent with a mechanism of action involving the loss of membrane integrity and function.

Antifungal Activity

Comprehensive investigations of the susceptibility of fungi to TTO have only recently been completed. Prior to this, data were somewhat piecemeal and fragmentary. Early data were also largely limited to *Candida albicans*, which was a commonly chosen model test organism. Data now show that a range of yeasts, dermatophytes, and other filamentous fungi are susceptible to TTO (14, 42, 52, 61, 116, 128, 140) (Table 4). Although

test methods differ, MICs generally range between 0.03 and 0.5%, and fungicidal concentrations generally range from 0.12 to 2%. The notable exception is *Aspergillus niger*, with minimal fungicidal concentrations (MFCs) of as high as 8% reported for this organism (74). However, these assays were performed with fungal conidia, which are known to be relatively impervious to chemical agents. Subsequent assays have shown that germinated conidia are significantly more susceptible to TTO than nongerminated conidia (74), suggesting that the intact conidial wall confers considerable protection. TTO vapors have also been demonstrated to inhibit fungal growth (86, 87) and affect sporulation (88).

Mechanism of antifungal action. Studies investigating the mechanism(s) of antifungal action have focused almost exclusively on *C. albicans*. Similar to results found for bacteria, TTO also alters the permeability of *C. albicans* cells. The treatment of *C. albicans* with 0.25% TTO resulted in the uptake of propidium iodide after 30 min (50), and after 6 h significant staining with methylene blue and loss of 260-nm-light-absorbing materials had occurred (72). TTO also alters the permeability of *Candida glabrata* (72). Further research demonstrating that the membrane fluidity of *C. albicans* cells treated with 0.25% TTO is significantly increased confirms that the oil substantially alters the membrane properties of *C. albicans* (72).

TTO also inhibits respiration in *C. albicans* in a dose-dependent manner (49). Respiration was inhibited by approximately 95% after treatment with 1.0% TTO and by approximately 40% after treatment with 0.25% TTO. The respiration rate of *Fusarium solani* is inhibited by 50% at a concentration of 0.023% TTO (88). TTO also inhibits glucose-induced medium acidification by *C. albicans*, *C. glabrata*, and *Saccharomyces cerevisiae* (72). Medium acidification occurs largely by the expulsion of protons by the plasma membrane ATPase, which is fuelled by ATP derived from the mitochondria. The inhibition of this function suggests that the plasma and/or mitochondrial membranes have been adversely affected. These results are consistent with a proposed mechanism of antifungal action whereby TTO causes changes or damage to the functioning of fungal membranes. This proposed mechanism is further supported by work showing that the terpene eugenol inhibits mitochondrial respiration and energy production (46).

Additional studies have shown that when cells of *C. albicans* are treated with diethylstilbestrol to inhibit the plasma membrane ATPase, they then have a much greater susceptibility to TTO than do control cells (72), suggesting that the plasma membrane ATPase has a role in protecting cells against the destabilizing or lethal effects of TTO.

TTO inhibits the formation of germ tubes, or mycelial conversion, in *C. albicans* (52, 78). Two studies have shown that germ tube formation was completely inhibited in the presence of 0.25 and 0.125% TTO, and it was further observed that treatment with 0.125% TTO resulted in a trend of blastospores changing from single or singly budding morphologies to multiply budding morphologies over the 4-h test period (78). These cells were actively growing but were not forming germ tubes, implying that morphogenesis is specifically inhibited, rather than all growth being inhibited. Interestingly, the inhibition of germ tube formation was shown to be reversible, since cells were able to form germ tubes after the removal of the

TTO (78). However, there was a delay in germ tube formation, indicating that TTO causes a postantifungal effect.

Antiviral Activity

The antiviral activity of TTO was first shown using tobacco mosaic virus and tobacco plants (18). In field trials with *Nicotiana glutinosa*, plants were sprayed with 100, 250, or 500 ppm TTO or control solutions and were then experimentally infected with tobacco mosaic virus. After 10 days, there were significantly fewer lesions per square centimeter of leaf in plants treated with TTO than in controls (18). Next, Schnitzler et al. (132) examined the activity of TTO and eucalyptus oil against herpes simplex virus (HSV). The effects of TTO were investigated by incubating viruses with various concentrations of TTO and then using these treated viruses to infect cell monolayers. After 4 days, the numbers of plaques formed by TTO-treated virus and untreated control virus were determined and compared. The concentration of TTO inhibiting 50% of plaque formation was 0.0009% for HSV type 1 (HSV-1) and 0.0008% for HSV-2, relative to controls. These studies also showed that at the higher concentration of 0.003%, TTO reduced HSV-1 titers by 98.2% and HSV-2 titers by 93.0%. In addition, by applying TTO at different stages in the virus replicative cycle, TTO was shown to have the greatest effect on free virus (prior to infection of cells), although when TTO was applied during the adsorption period, a slight reduction in plaque formation was also seen (132). Another study evaluated the activities of 12 essential oils, including TTO, for activity against HSV-1 in Vero cells (110). Again, TTO was found to exert most of its antiviral activity on free virus, with 1% oil inhibiting plaque formation completely and 0.1% TTO reducing plaque formation by approximately 10%. Pretreatment of the Vero cells prior to virus addition or posttreatment with 0.1% TTO after viral absorption did not significantly alter plaque formation.

Some activity against bacteriophages has also been reported, with exposure to 50% TTO at 4°C for 24 h reducing the number of SA and T7 plaques formed on lawns of *S. aureus* and *E. coli*, respectively (41).

The results of these studies indicate that TTO may act against enveloped and nonenveloped viruses, although the range of viruses tested to date is very limited.

Antiprotozoal Activity

Two publications show that TTO has antiprotozoal activity. TTO caused a 50% reduction in growth (compared to controls) of the protozoa *Leishmania major* and *Trypanosoma brucei* at concentrations of 403 mg/ml and 0.5 mg/ml, respectively (109). Further investigation showed that terpinen-4-ol contributed significantly to this activity. In another study, TTO at 300 mg/ml killed all cells of *Trichomonas vaginalis* (151). There is also anecdotal in vivo evidence that TTO may be effective in treating *Trichomonas vaginalis* infections (120).

Antimicrobial Components of TTO

Considerable attention has been paid to which components of TTO are responsible for the antimicrobial activity. Early

indications from RW coefficients were that much of the activity could be attributed to terpinen-4-ol and α -terpineol (121). Data available today confirm that these two components contribute substantially to the oil's antibacterial and antifungal activities, with MICs and MBCs or MFCs that are generally the same as, or slightly lower than values obtained for TTO (36, 42, 48, 71, 117, 126). However, α -terpineol does not represent a significant proportion of the oil. Additional components with MICs similar to or lower than those of TTO include α -pinene, β -pinene, and linalool (36, 71), but, similar to the case for α -terpineol, these components are present in only relatively low amounts. Of the remaining components tested, it seems that most possess at least some degree of antimicrobial activity (36, 71, 126), and this is thought to correlate with the presence of functional groups, such as alcohols, and the solubility of the component in biological membranes (63, 138). While some TTO components may be considered less active, none can be considered inactive. Furthermore, methodological issues have been demonstrated to have a significant influence on assay outcomes (48, 71).

The possibility that components in TTO may have synergistic or antagonistic interactions has been explored in vitro (48), but no significant relationships were found. The possibility that TTO may act synergistically with other essential oils, such as lavender (38), and other essential oil components, such as β -triketones from manuka oil (43, 44), has also been investigated. Given the numerous components of TTO, the scope for such effects is enormous, and much more work is required to examine this question.

Resistance to TTO

The question of whether true resistance to TTO can be induced in vitro or may occur spontaneously in vivo has not been examined systematically. Clinical resistance to TTO has not been reported, despite the medicinal use of the oil in Australia since the 1920s. There has been one short report of induced in vitro resistance to TTO in *S. aureus* (114). Stepwise exposure of five MRSA isolates to increasing concentrations of TTO yielded three isolates with TTO MICs of 1% and one isolate each with TTO MICs of 2% and 16%, respectively. All isolates showed initial MICs of 0.25%. There has also been one report suggesting that *E. coli* strains harboring mutations in the multiple antibiotic resistance (*mar*) operon, so-called Mar mutants, may exhibit decreased susceptibility to TTO (66). Minor changes in TTO and α -terpineol susceptibilities have also been seen in *S. aureus* isolates with reduced susceptibility to household cleaners (53). However, in these last two studies the changes in susceptibility were marginal and do not represent strong evidence of resistance (53, 66). With regard to fungi, an attempt to induce resistance to TTO in two clinical isolates of *Candida albicans* was largely unsuccessful, with isolates failing to grow in 2% (vol/vol) TTO after serial passage in increasing concentrations of TTO (111).

Resistance to conventional antibiotics has not been demonstrated to influence susceptibility to TTO, suggesting that cross-resistance does not occur. For example, antimicrobial-resistant isolates of *S. aureus* (31, 58), *C. albicans* and *C. glabrata* (148), *P. aeruginosa* (106), and *Enterococcus faecium*

(106, 115) have *in vitro* susceptibilities to TTO that are similar to those of nonresistant isolates.

Overall, these studies provide little evidence to suggest that resistance to TTO will occur, either *in vitro* or *in vivo*, although minimal data are available. It is likely that the multicomponent nature of TTO may reduce the potential for resistance to occur spontaneously, since multiple simultaneous mutations may be required to overcome all of the antimicrobial actions of each of the components. Furthermore, since TTO is known to affect cell membranes, it presumably affects multiple properties and functions associated with the cell membrane, similar to the case for membrane-active biocides. This means that numerous targets would have to adapt to overcome the effects of the oil. Issues of potential resistance are important if TTO is to be used more widely, particularly against antibiotic-resistant organisms.

CLINICAL EFFICACY

In parallel with the characterization of the *in vitro* antimicrobial activity of TTO, the clinical efficacy of the oil has also been the subject of investigation. Early clinical studies attempting to characterize the clinical efficacy of TTO (60, 120, 152) are not considered scientifically valid by today's standards and will therefore not be discussed further. Data from some of the more recent clinical investigations are summarized in Table 5.

One of the first rigorous clinical studies assessed the efficacy of 5% TTO in the treatment of acne by comparing it to 5% benzoyl peroxide (BP) (14). The study found that both treatments reduced the numbers of inflamed lesions, although BP performed significantly better than TTO. The BP group showed significantly less oiliness than the TTO group, whereas the TTO group showed significantly less scaling, pruritis, and dryness. Significantly fewer overall side effects were reported by the TTO group (27 of 61 patients) than by the BP group (50 of 63 patients).

The efficacy of TTO in dental applications has been assessed. An evaluation of the effect of a 0.2% TTO mouthwash and two other active agents on the oral flora of 40 volunteers suggested that TTO used once daily for 7 days could reduce the number of mutants streptococci and the total number of oral bacteria, compared to placebo treatment. The data also indicated that these reductions were maintained for 2 weeks after the use of mouthwash ceased (64). In another study, comparison of mouthwashes containing either approximately 0.34% TTO, 0.1% chlorhexidine, or placebo on plaque formation and vitality, using eight volunteers (9), showed that after TTO treatment, both plaque index and vitality did not differ from those of subjects receiving placebo mouthwash on any day, whereas the results for the chlorhexidine mouthwash group differed significantly from those for the placebo group on all days (9). Lastly, a study comparing a 2.5% TTO gel, a 0.2% chlorhexidine gel, and a placebo gel found that although the TTO group had significantly reduced gingival index and papillary bleeding index scores, their plaque scores were actually increased (139). These studies indicate that although TTO may cause decreases in the levels of oral bacteria, this does not necessarily equate to reduced plaque levels. However, TTO may have a role in the treatment of gingivitis, and there is also

some evidence preliminary suggesting that TTO reduces the levels of several compounds associated with halitosis (144).

Two studies have assessed the efficacy of TTO for the eradication of MRSA carriage. The effectiveness of a 4% TTO nasal ointment and a 5% TTO body wash was compared to that of conventional treatment with mupirocin nasal ointment and Triclosan body wash in a small pilot study (28). Of the 15 patients receiving conventional treatment, 2 were cleared and 8 remained colonized at the end of therapy; in the TTO group of 15, 5 were cleared and 3 remained colonized. The remainder of patients did not complete therapy. Differences in clearance rates were not statistically significant, most likely due to the low patient numbers. Stronger evidence for the efficacy of TTO in decolonizing MRSA carriage comes from a recent trial in which 236 patients were randomized to receive standard or TTO treatment regimens (56). The standard regimen consisted of 2% mupirocin nasal ointment applied three times a day, 4% chlorhexidine gluconate soap applied at least once a day, and 1% silver sulfadiazine cream applied to skin lesions, wounds, and leg ulcers once a day, all for 5 days. The TTO regimen consisted of 10% TTO nasal cream applied three times a day, 5% TTO body wash applied at least once daily and 10% TTO cream applied to skin lesions, wounds, and leg ulcers once a day, all for 5 days. The 10% TTO cream was allowed to be used as an alternative to the body wash. Follow-up swabs were taken at 2 and 14 days posttreatment, with the exception of 12 patients who were lost to follow-up. Evaluation of the remaining 224 patients showed no significant differences between treatment regimens, with 49% of patients receiving standard therapy cleared versus 41% of patients in the TTO group.

For many years there has been considerable interest in the possibility of using TTO in handwash formulations for use in hospital or health care settings. It is well known that handwashing is an effective infection control measure and that lack of compliance is related to increased rates of nosocomial infections. The benefits of using TTO in a handwash formulation include its antiseptic effects and increased handwashing compliance. A recent handwash study using volunteers showed that either a product containing 5% TTO and 10% alcohol or a solution of 5% TTO in water performed significantly better than soft soap, whereas a handwash product containing 5% TTO did not (108).

Occasional case reports of the use of TTO have also been published. In one, a woman self-treated successfully with a 5-day course of TTO pessaries after having been clinically diagnosed with bacterial vaginosis (19). In a second, a combination of plant extracts of which TTO was a major component was inserted percutaneously into bone to treat an intractable MRSA infection of the lower tibia, which subsequently resolved (136). This same essential oil solution has now been shown to aid in the healing of malodorous malignant ulcers (154).

With regard to fungal infections, TTO has been clinically evaluated for the treatment of onychomycosis (26, 143), tinea pedis (131, 145), dandruff (130), and oral candidiasis (92, 149). Although much has been made of the potential for TTO to be used in the treatment of vaginal candidiasis, no clinical data have been published. However, results from an animal (rat) model of vaginal candidiasis support the use of TTO for the treatment of this infection (111).

In the first of the onychomycosis trials (26), 60% of patients

TABLE 5. Summary of clinical studies using TTO

Study population	Study type	Treatment groups (no. of evaluable patients)	Administration of treatment	Outcomes	Adverse events	Reference
124 patients with mild to moderate acne	RCT*, investigator-blinded ^a	5% TTO gel (58), 5% benzoyl peroxide (61)	3 mo	Both significantly reduced inflamed lesions ($P < 0.001$) but BP better at reducing whiteheads ($P < 0.05$), BP better at reducing comedones ($P < 0.002$), less redness ($P < 0.001$), less dryness ($P < 0.001$) with TTO; treatments equivalent for noninflamed lesions, erythema Median time to repopularization of 9 days for TTO vs 12.5 days for benzoyl peroxide	27 (44%) in TTO group, 50 (79%) in BP group (c.g., dryness, stinging, burning, redness); significantly fewer events in TTO group ($P < 0.001$)	14
18 patients with recurrent herpes labialis (cold sores)	RCT, investigator-blinded ^a	6% TTO gel (9), placebo gel (9)	5 times daily	Whole scalp lesion score significantly improved in TTO group (4.2%) compared to placebo group (11.2%) ($P < 0.001$)	1 in TTO group (event not stated)	30
126 patients with mild to moderate dandruff	RCT, investigator-blinded ^a	5% TTO shampoo (62), placebo shampoo (62)	Daily for 4 wk	For TTO, 53% cleared, 29% chronic, 18% nonresponders; for placebo, 3% cleared, 53% chronic, 35% incoincident (no significant differences)	3 (6%) in TTO group, 8 (13%) in placebo group (c.g., mild burning, stinging, itching)	130
30 hospital inpatients colonized or infected with MRSA	Randomized, controlled pilot study	4% TTO nasal ointment + 5% TTO body wash (15), 2% Triclosan nasal ointment + 4% Triclosan body wash (15), 2% Triclosan sulfadiazine cream (14)	Frequency not stated, minimum of 3 days	For TTO, 53% cleared, 29% chronic, 18% nonresponders; for placebo, 3% cleared, 53% chronic, 35% incoincident (no significant differences)	With TTO nasal ointment (too, not stated), topical use of nasal mucosa to acute burning	28
236 hospital patients colonized with MRSA	RCT	10% TTO cream + 5% TTO body wash (110), 2% Triclosan nasal ointment + 4% Triclosan body wash + 1% silver sulfadiazine cream (14)	Once daily for 3 days	For TTO, 41% cleared, for treatment regimens did not differ significantly ($P = 0.0286$); mupirocin significantly better than TTO at clearing nasal carriage ($P = 0.0001$)	None	50
117 patients with culture-positive onychomycosis	RCT, double-blind	100% TTO (64), 1% dermatone (53)	Twice daily for 6 mo	Full or partial resolution for 60% of TTO and 61% of dermatone (not significant; $P > 0.05$)	5 (7.8%) in TTO group, 3 (5.7%) in dermatone group (erythema, irritation, edema)	26
60 outpatients with a clinical diagnosis of 13 patients with AIDS and fluconazole-refractory oral candidiasis	RCT, double-blind Crossover	2% butenafine hydrochloride with 5% TTO cream (40), 5% TTO cream (20) Miconazole solution (15 ml) (12)	3 times daily for 8 wk 4 times daily for 2-4 wk	Cure in 86% of butenafine/TTO group and 0% of TTO group ($P < 0.0001$)	4 (10%) in butenafine/TTO group (mild inflammation)	143
27 patients with AIDS and fluconazole-refractory oral candidiasis	Open-label trial	Melatonin and solution (15 ml) (12), alcohol-free solution and solution (5 ml) (13)	4 times daily for 2-4 wk	Clinical response rate of 67% after 4 weeks in 13 patients, no response in 4 patients.	None	92
121 patients with culture-positive tinea pedis	RCT, double-blind	10% TTO in acetone (37), 1% butenafine (33), placebo (acetone) (34)	Twice daily for 4 wk	Mycopegical and clinical response in 54% (butenafine solution) and 54% (placebo solution) of patients after 4 wk	8 (66.7%) in alcohol-based solution group, 2 (15.4%) in alcohol-free solution group (mild to moderate burning)	149
137 patients with culture-positive tinea pedis	RCT, double-blind	25% TTO (36), 50% TTO (38), placebo (46)	Twice daily for 4 wk	Improvement in 46% (butenafine), 22% (TTO), and 9% (placebo) of patients; butenafine significantly better than placebo ($P < 0.001$) but not TTO ($P = 0.59$); TTO not different from placebo ($P = 0.3$)	None	145

^a RCT, randomized controlled trial^b The distinctive odor of TTO was stated as preventing patient blinding^c The alcohol-free solution was more concentrated, and thus a smaller volume was used.

treated with TTO and 61% of patients treated with 1% clotrimazole had full or partial resolution. There were no statistically significant differences between the two treatment groups for any parameter. The second onychomycosis trial (143) compared two creams, one containing 5% TTO alone and the other containing 5% TTO and 2% butenafine, both applied three times daily for 8 weeks. The overall cure rate was 0% for patients treated with 5% TTO alone, compared to 80% for patients treated with both butenafine and TTO. Unfortunately, butenafine alone was not evaluated. The observation that TTO may be useful adjunct therapy for onychomycosis has been made by Klimmek et al. (95). However, onychomycosis is considered to be largely unresponsive to topical treatment of any kind, and a high rate of cure should therefore not be expected.

The effectiveness of TTO in treating tinea pedis has been evaluated in two trials. In the first trial, patients were treated with 10% TTO in sorbolene, 1% tolnaftate, or placebo (sorbolene) (145). At completion of treatment, patients treated with TTO had mycological cure and clinical improvement rates of 30% and 65%, respectively. This compares to mycological cure rates of 21% in patients receiving placebo and 85% in patients receiving tolnaftate. Similarly, clinical improvement was seen in 41% of patients receiving placebo and 68% of patients receiving tolnaftate. In a second tinea trial, the efficacy of solutions of 25% and 50% TTO in ethanol and polyethylene glycol was compared to treatment with placebo (vehicle) (131). Marked clinical responses were seen in 72% and 68% of patients in the 25% and 50% TTO treatment groups, respectively, compared to 39% of patients in the placebo group. Similarly, there were mycological cures of 55% and 64% in the 25% and 50% TTO treatment groups, respectively, compared to 31% in the placebo group. Dermatitis occurred in one patient in the 25% TTO group and in three patients in the 50% TTO group. This led to the recommendation that 25% TTO be considered an alternative treatment for tinea, since it was associated with fewer adverse reactions than but was just as effective as 50% TTO. These studies highlight the importance of considering the formulation of the TTO product when conducting *in vivo* work, since it is likely that the sorbolene vehicle used in the first tinea trial may have significantly compromised the antifungal activity of the oil.

The evaluation of a 5% TTO shampoo for mild to moderate dandruff demonstrated statistically significant improvements in the investigator-assessed whole scalp lesion score, total area of involvement score, and total severity score, as well as in the patient-assessed itchiness and greasiness scores, compared to placebo. Overall, the 5% TTO was well tolerated and appeared to be effective in the treatment of mild to moderate dandruff.

TTO has been evaluated as a mouthwash in the treatment of oropharyngeal candidiasis. In a case series, 13 human immunodeficiency virus-positive patients who had already failed treatment with a 14-day course of oral fluconazole were treated with an alcohol-based TTO solution for up to 28 days (92). After treatment, of the 12 evaluable patients, 2 were cured, 6 were improved, 4 were unchanged, and 1 had deteriorated. Overall, eight patients had a clinical response and seven had a mycological response. In subsequent work the same TTO solution was compared with an alcohol-free TTO solution (149). Of patients receiving the alcohol-based solution, two were cured, six improved, four were unchanged, and

one had deteriorated. Of patients receiving the alcohol-free solution, five were cured, two improved, two were unchanged, and one had deteriorated. Three patients were lost to follow-up and were considered nonresponders.

Support for TTO possessing *in vivo* antiviral activity comes from a pilot study investigating the treatment of recurrent herpes labialis (cold sores) with a 6% TTO gel or a placebo gel (30). Comparison of the patient groups (each containing nine evaluable patients) at the end of the study showed that reepithelialization after treatment occurred after 9 days for the TTO group and after 12.5 days for the placebo group. Other measures, such as duration of virus positivity by culture or PCR, viral titers, and time to crust formation, were not significantly different, possibly due to small patient numbers. Interestingly, when TTO was evaluated for its protective efficacy in an *in vivo* mouse model of genital HSV type 2 infection, it did not perform well (21). In contrast, the oil component 1,8-cineole performed well, protecting 7 of 16 animals from disease.

There are a number of limitations to the clinical studies described above. Several had low numbers of participants, meaning that statistical analyses could not be performed or differences did not reach significance. Many studies had ambiguous and/or equivocal outcomes. Of those studies with larger numbers of patients, few reported 95% confidence intervals or relative risk values. While most studies compared the efficacy of TTO to a placebo, many did not compare TTO to a conventional therapy or treatment regimen, again limiting the conclusions that could be drawn about efficacy. Several publications noted that patient blinding was compromised or impracticable due to the characteristic odor of TTO (14, 30, 130, 131). These studies, while perhaps conducted as double blinded, are technically only single blinded, which is not ideal. Perhaps most importantly, few studies have been replicated independently. Therefore, although some of these data indicate that TTO has potential as a therapeutic agent, confirmatory studies are required. In addition, factors such as the final TTO concentration, product formulation, and length and frequency of treatment undoubtedly influence clinical efficacy, and these factors must be considered in future studies. The cost-effectiveness of any potential TTO treatments must also be considered. For example, TTO therapy may offer no cost advantage over the azoles in the treatment of tinea but is probably more economical than treatment with the allylamines.

ANTI-INFLAMMATORY ACTIVITY

Numerous recent studies now support the anecdotal evidence attributing anti-inflammatory activity to TTO. *In vitro* work over the last decade has demonstrated that TTO affects a range of immune responses, both *in vitro* and *in vivo*. For example, the water-soluble components of TTO can inhibit the lipopolysaccharide-induced production of the inflammatory mediators tumor necrosis factor α (TNF- α), interleukin-1 β (IL-1 β), and IL-10 by human peripheral blood monocytes by approximately 50% and that of prostaglandin E_2 by about 30% after 40 h (81). Further examination of the water-soluble fraction of TTO identified terpinen-4-ol, α -terpineol, and 1,8-cineole as the main components, but of these, only terpinen-4-ol was able to diminish the production of TNF- α , IL-1 β , IL-8, IL-10, and prostaglandin E_2 by lipopolysaccharide-activated

monocytes. The water-soluble fraction of TTO, terpinen-4-ol, and α -terpineol also suppressed superoxide production by agonist-stimulated monocytes but not neutrophils (22). In contrast, similar work found that TTO decreases the production of reactive oxygen species by both stimulated neutrophils and monocytes and that it also stimulates the production of reactive oxygen species by nonprimed neutrophils and monocytes (29). TTO failed to suppress the adherence reaction of neutrophils induced by TNF- α stimulation (2) or the casein-induced recruitment of neutrophils into the peritoneal cavities of mice (1). These studies identify specific mechanisms by which TTO may act in vivo to diminish the normal inflammatory response. In vivo, topically applied TTO has been shown to modulate the edema associated with the efferent phase of a contact hypersensitivity response in mice (23) but not the development of edema in the skin of nonsensitized mice or the edematous response to UVB exposure. This activity was attributed primarily to terpinen-4-ol and α -terpineol. When the effect of TTO on hypersensitivity reactions involving mast cell degranulation was examined in mice, TTO and terpinen-4-ol applied after histamine injection reduced histamine-induced skin edema, and TTO also significantly reduced swelling induced by intradermal injection of compound 48/80 (24). Human studies on histamine-induced wheal and flare provided further evidence to support the in vitro and animal data, with the topical application of neat TTO significantly reducing mean wheal volume but not mean flare area (97). Erythema and flare associated with nickel-induced contact hypersensitivity in humans are also reduced by neat TTO but not by a 5% TTO product, product base, or macadamia oil (119). Work has now shown that terpinen-4-ol, but not 1,8-cineole or α -terpineol, modulates the vasodilation and plasma extravasation associated with histamine-induced inflammation in humans (94).

SAFETY AND TOXICITY

Despite the progress in characterizing the antimicrobial and anti-inflammatory properties of tea tree oil, less work has been done on the safety and toxicity of the oil. The rationale for continued use of the oil rests largely on the apparently safe use of the oil for almost 80 years. Anecdotal evidence over this time suggests that topical use is safe and that adverse events are minor, self-limiting, and infrequent. More concrete evidence such as published scientific work is scarce, and much information remains out of the public domain in the form of reports from company-sponsored work. The oral and dermal toxicities of TTO are summarized briefly below.

Oral Toxicity

TTO can be toxic if ingested, as evidenced by studies with animals and from cases of human poisoning. The 50% lethal dose for TTO in a rat model is 1.9 to 2.6 ml/kg (129), and rats dosed with ≤ 1.5 g/kg TTO appeared lethargic and ataxic (D. Kim, D. R. Cerven, S. Craig, and G. L. De George, Abstr. Amer. Chem. Soc. 223:114, 2002). Incidences of oral poisoning in children (55, 91, 112) and adults (57, 133) have been reported. In all cases, patients responded to supportive care and recovered without apparent sequelae. No human deaths due to TTO have been reported in the literature.

Dermal Toxicity

TTO can cause both irritant and allergic reactions. A mean irritancy score of 0.25 has been found for neat TTO, based on patch testing results for 311 volunteers (10). Another study, in which 217 patients from a dermatology clinic were patch tested with 10% TTO, found no irritant reactions (150). Since irritant reactions may frequently be avoided through the use of lower concentrations of the irritant, this bolsters the case for discouraging the use of neat oil and promoting the use of well-formulated products. Allergic reactions have been reported (54, 147), and although a range of components have been suggested as responsible, the most definitive work indicates that they are caused mainly by oxidation products that occur in aged or improperly stored oil (82). There is little scientific support for the notion that 1,8-cineole is the major irritant in TTO. No evidence of irritation was seen when patch testing was performed on rabbits with intact and abraded skin (118), guinea pigs (82), and humans (118, 141), including those who had previous positive reactions to TTO (96). Rarely, topically applied tea tree oil has been reported to cause systemic effects in domestic animals. Dermal application of approximately 120 ml of undiluted TTO to three cats with shaved but intact skin resulted in symptoms of hypothermia, uncoordination, dehydration, and trembling and in the death of one of the cats (17).

PRODUCT FORMULATION ISSUES

The physical characteristics of TTO present certain difficulties for the formulation and packaging of products. Its lipophilicity leads to miscibility problems in water-based products, while its volatility means that packaging must provide an adequate barrier to volatilization. Since TTO is readily absorbed into plastics, packaging must cater to and minimize this effect. Consideration must also be given to the properties of the finished product. Early suggestions that the antimicrobial activity of TTO may be compromised by organic matter came from disk diffusion studies in which the addition of blood to agar medium decreased zone sizes (8). This observation contrasts sharply with historical claims that the activity of TTO may in fact be enhanced in the presence of organic matter such as blood and pus. A thorough investigation of this claim comprehensively refuted this idea (76) and also showed that product excipients may compromise activity.

Some work on the characteristics and behavior of TTO within formulations has been conducted. Caboi et al. (27) examined the potential of a monolcin/water system as a carrier for TTO and terpinen-4-ol. The activity of TTO products in vitro has also been investigated (16, 77, 107). However, very little work has been conducted in this area, and if stable, biologically active formulations of TTO are going to be developed, much remains to be done.

CONCLUSIONS

A paradigm shift in the treatment of infectious diseases is necessary to prevent antibiotics becoming obsolete, and where appropriate, alternatives to antibiotics ought to be considered. There are already several nonantibiotic approaches to the treatment and prevention of infection, including probiotics,

phages, and phytomedicines. Alternative therapies are viewed favorably by many patients because they are often not being favored by conventional therapy and they believe there are fewer detrimental side effects. In addition, many report significant improvement while taking complementary and alternative medicines. Unfortunately, the medical profession has been slow to embrace these therapies, and good scientific data are still scarce. However, as we approach the "postantibiotic era" the situation is changing. A wealth of *in vitro* data now supports the long-held beliefs that TTO has antimicrobial and anti-inflammatory properties. Despite some progress, there is still a lack of clinical evidence demonstrating efficacy against bacterial, fungal, or viral infections. Large randomized clinical trials are now required to cement a place for TTO as a topical medicinal agent.

ACKNOWLEDGMENTS

This review was supported in part by a grant (UWA-75A) from the Rural Industries Research and Development Corporation.

We are grateful to Ian Southwell (Wollongong Agricultural Institute, NSW) for helpful discussions on oil provenance and to staff at the Australian War Memorial (Canberra, ACT) for sharing their knowledge of Australian military history and TTO.

REFERENCES

1. Abe, S., N. Maruyama, K. Hayama, S. Inoue, H. Oshima, and H. Yamaguchi. 2004. Suppression of neutrophil recruitment in mice by geranium essential oil. *Med. Inflamm.* 13:21-24.
2. Abe, S., N. Maruyama, K. Hayama, H. Ishibashi, S. Inoue, H. Oshima, and H. Yamaguchi. 2003. Suppression of tumor necrosis factor- α -induced neutrophil adherence responses by essential oils. *Med. Inflamm.* 12:323-328.
3. Altman, P. M. 1988. Australian tea tree oil. *Aust. J. Pharm.* 69:276-278.
4. Andrews, R., E. W. Parks, and K. D. Spence. 1980. Some effects of Douglas fir terpenes on certain microorganisms. *Appl. Environ. Microbiol.* 40:301-304.
5. Anonymous. 1933. An Australian antiseptic oil. *Br. Med. J.* 1:966.
6. Anonymous. 1930. A retrospect. *Med. J. Aust.* 1:85-89.
7. Anonymous. 1933. T-sterol oil. *Br. Med. J.* 1:1927.
8. Ansteth, S. 1990. The effect of tea tree oil on human pathogenic bacteria and fungi in a laboratory study. *Swed. J. Biol. Med.* 2:3-8.
9. Arweiler, R. B., N. Donus, L. Neuschil, K. Reich, and A. Seilman. 2000. Clinical and antibacterial effect of tea tree oil—a pilot study. *Clin. Oral Investig.* 4:70-73.
10. Aspres, N., and S. Freeman. 2003. Predictive testing for irritancy and allergenicity of tea tree oil in normal human subjects. *Exogenous Dermatol.* 2:258-261.
11. Aikman, N., and H. E. Brice. 1955. Antibacterial substances produced by flowering plants. *Australas. J. Exp. Biol.* 33:547-554.
12. Baker, G. 1999. Tea tree breeding. p. 135-154. In I. Southwell and R. Lowe (ed.), *Tea tree: the genus Melaleuca*, vol. 9. Harwood Academic Publishers, Amsterdam, The Netherlands.
13. Bancs-Marshall, L., P. Cawley, and A. C. Phillips. 2001. *In vitro* activity of *Melaleuca alternifolia* (tea tree) oil against bacterial and *Candida* spp. isolates from clinical specimens. *Br. J. Biomed. Sci.* 58:139-145.
14. Bassett, I. B., D. L. Pannowitz, and R. S. Barnett. 1990. A comparative study of tea-tree oil versus benzoylperoxide in the treatment of acne. *Med. J. Aust.* 153:455-458.
15. Beylitz, M. F. 1979. Bacteriostatic activity of some Australian essential oils. *Perflav.* Flavourist 4:23-25.
16. Bijle, S. S., A. Ahuja, R. K. Khar, and R. Chaudhry. 2005. Formulation and evaluation of an effective pH balanced topical antimicrobial product containing tea tree oil. *Pharmazie* 60:208-211.
17. Blachoff, K., and F. Gnani. 1998. Australian tea tree (*Melaleuca alternifolia*) oil poisoning in three purchased cases. *J. Vet. Diagn. Investig.* 10:208-210.
18. Bishop, C. D. 1995. Antiviral activity of the essential oil of *Melaleuca alternifolia* (Maiden & Betche) Cheal (tea tree) against tobacco mosaic virus. *J. Essent. Oil Res.* 7:641-644.
19. Blackwell, A. L. 1991. Tea tree oil and anaerobic (bacterial) vaginosis. *Lancet* 337:300.
20. Blackwell, R. 1991. An insight into aromatic oils: lavender and tea tree. *Br. J. Phytother.* 2:26-30.
21. Bourne, K. Z., N. Bourne, S. F. Reising, and L. R. Stanberry. 1999. Plant products as topical microbicide candidates: assessment of *in vitro* and *in vivo* activity against herpes simplex virus type 2. *Antiviral Res.* 42:219-226.
22. Brand, C., A. Ferrante, R. H. Prager, T. V. Riley, C. F. Carson, J. J. Finlay-Jones, and P. H. Hart. 2001. The water soluble-components of the essential oil of *Melaleuca alternifolia* (tea tree oil) suppress the production of superoxide by human monocytes, but not neutrophils, activated *in vitro*. *Inflamm. Res.* 50:213-219.
23. Brand, C., M. A. Grimbaldeston, J. R. Gamble, J. Drew, J. J. Finlay-Jones, and P. H. Hart. 2002. Tea tree oil reduces the swelling associated with the efferent phase of a contact hypersensitivity response. *Inflamm. Res.* 51:236-244.
24. Brand, C., S. L. Townley, J. J. Finlay-Jones, and P. H. Hart. 2002. Tea tree oil reduces histamine-induced oedema in murine ears. *Inflamm. Res.* 51:283-289.
25. Brophy, J. J., N. W. Davies, I. A. Southwell, I. A. Stiff, and L. R. Williams. 1989. Gas chromatographic quality control for oil of *Melaleuca terpinen-4-ol* type (Australian tea tree). *J. Agric. Food Chem.* 37:1330-1335.
26. Beck, D. S., D. M. Nidford, and J. G. Addine. 1994. Comparison of two topical preparations for the treatment of onychomycosis: *Melaleuca alternifolia* (tea tree) oil and clotrimazole. *J. Fam. Pract.* 38:601-605.
27. Cabot, F., S. Murgia, M. Mondazzi, and P. Lazzari. 2002. NMR investigation on *Melaleuca alternifolia* essential oil displaced in the monodentate aqueous system: phase behavior and dynamics. *Langmuir* 18:7916-7922.
28. Castelli, M., J. Porteous, C. F. Carson, R. Heiler, and T. V. Riley. 2000. Tea tree oil as an alternative topical decolonization agent for methicillin-resistant *Staphylococcus aureus*. *J. Hosp. Infect.* 46:236-237.
29. Caldeffe-Chazet, F., M. Guerry, J. C. Chalchot, C. Fissillier, M. P. Vasson, and J. Guillet. 2004. Anti-inflammatory effects of *Melaleuca alternifolia* essential oil on human polymorphonuclear neutrophils and monocytes. *Free Rad. Res.* 38:805-811.
30. Carson, C. F., L. Ashton, L. Dry, D. W. Smith, and T. V. Riley. 2001. *Melaleuca alternifolia* (tea tree) oil gel (6%) for the treatment of recurrent herpes labialis. *J. Antimicrob. Chemother.* 48:450-451.
31. Carson, C. F., B. D. Cookson, H. D. Farrelly, and T. V. Riley. 1995. Susceptibility of methicillin-resistant *Staphylococcus aureus* to the essential oil of *Melaleuca alternifolia* (tea tree oil). *Microbios* 82:181-185.
32. Carson, C. F., K. A. Hammer, and T. V. Riley. 1995. Broth micro-dilution method for determining the susceptibility of *Escherichia coli* and *Staphylococcus aureus* to the essential oil of *Melaleuca alternifolia* (tea tree oil). *Microbios* 82:181-185.
33. Carson, C. F., K. A. Hammer, and T. V. Riley. 1996. *In-vitro* activity of the essential oil of *Melaleuca alternifolia* against *Streptococcus* spp. *J. Antimicrob. Chemother.* 37:1177-1178.
34. Carson, C. F., B. J. Mos, and T. V. Riley. 2002. Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. *Antimicrob. Agents Chemother.* 46:1914-1920.
35. Carson, C. F., and T. V. Riley. 1993. Antimicrobial activity of the essential oil of *Melaleuca alternifolia*. *Lett. Appl. Microbiol.* 16:49-55.
36. Carson, C. F., and T. V. Riley. 1995. Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifolia*. *J. Appl. Bacteriol.* 78:264-269.
37. Carson, C. F., and T. V. Riley. 1994. Susceptibility of *Propionibacterium acnes* to the essential oil of *Melaleuca alternifolia*. *Lett. Appl. Microbiol.* 19:24-25.
38. Cassella, S., J. P. Cassella, and I. Smith. 2002. Synergistic antifungal activity of tea tree (*Melaleuca alternifolia*) and lavender (*Lawandula angustifolia*) essential oils against dermatophyte infection. *Int. J. Aromather.* 12:2-15.
39. Chan, C. H., and K. W. Loudon. 1998. Activity of tea tree oil on methicillin-resistant *Staphylococcus aureus* (MRSA). *J. Hosp. Infect.* 39:244-245.
40. Chand, S., I. Lajunen, D. A. Veal, L. R. Williams, and P. Caruso. 1994. Rapid screening of the antimicrobial activity of extracts and natural products. *J. Antibiot.* 47:1295-1304.
41. Chao, S. C., D. G. Young, and C. J. Oberg. 2000. Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. *J. Essent. Oil Res.* 12:639-649.
42. Christoph, F., P. M. Kaulfers, and E. Stahl-Biskup. 2000. A comparative study of the *in vitro* antimicrobial activity of tea tree oils s.l. with special reference to the activity of β -triketones. *Phanta Med.* 66:550-560.
43. Christoph, F., P. M. Kaulfers, and E. Stahl-Biskup. 2001. *In vitro* evaluation of the antimicrobial activity of β -triketones admixed to *Melaleuca* oils. *Phanta Med.* 67:768-771.
44. Christoph, F., E. Stahl-Biskup, and P. M. Kaulfers. 2001. Death kinetics of *Staphylococcus aureus* exposed to commercial tea tree oils s.l. *J. Essent. Oil Res.* 13:98-102.
45. Colton, R. T., and G. J. Murtagh. 1999. Cultivation of tea tree, p. 63-78. In I. Southwell and R. Lowe (ed.), *Tea tree: the genus Melaleuca*, vol. 9. Harwood Academic Publishers, Amsterdam, The Netherlands.
46. Cotman, J. M., A. Burke, N. H. Lee, and I. M. Shupler. 1979. Respiratory inhibition of isolated rat liver mitochondria by eugenol. *Arch. Oral Biol.* 24:565-568.
47. Cox, S. D., J. E. Gustafson, C. M. Mann, J. L. Markham, Y. C. Liew, R. J. Hartland, H. C. Bell, J. R. Warrington, and S. G. Wyllie. 1998. Tea tree oil

- causes K+ leakage and inhibits respiration in *Escherichia coli*. Lett. Appl. Microbiol. 26:353–358.
48. Cox, S. D., C. M. Mann, and J. L. Markham. 2001. Interactions between components of the essential oil of *Melaleuca alternifolia*. J. Appl. Microbiol. 91:492–497.
 49. Cox, S. D., C. M. Mann, J. L. Markham, H. C. Bell, J. E. Gustafson, J. R. Warrington, and S. G. Wyllie. 2000. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). J. Appl. Microbiol. 88:170–175.
 50. Cox, S. D., C. M. Mann, J. L. Markham, J. E. Gustafson, J. R. Warrington, and S. G. Wyllie. 2001. Determining the antimicrobial actions of tea tree oil. Molecules 6:87–91.
 51. Craven, L. A. 1999. Behind the names: the history of tea tree, cajuput and ninoöl, p. 11–28. In I. Southwell and R. Lowe (ed.), Tea tree: the genus *Melaleuca*, vol. 9. Harwood Academic Publishers, Amsterdam, The Netherlands.
 52. D'Auria, F. D., L. Laino, V. Strippoli, M. Tecca, G. Salvatore, L. Battinelli, and G. Muzantini. 2001. In vitro activity of tea tree oil against *Candida albicans* mycelial conversion and other pathogenic fungi. J. Chemother. 13:377–383.
 53. Davis, A., J. O'Leary, A. Muthaiyaz, M. Lungvin, A. Delgado, A. Abalos, A. Fajardo, J. Marek, B. Wilderson, and J. Gustafson. 2005. Characterization of *Staphylococcus aureus* isolates expressing reduced susceptibility to common house-keepers. J. Appl. Microbiol. 98:364–372.
 54. De Groot, A. C., and J. W. Weijland. 1992. Systemic contact dermatitis from tea tree oil. Contact Dermatitis 27:279–280.
 55. Del Beccaro, M. A. 1995. Melaleuca oil poisoning in a 17-month-old. Vet. Hum. Toxicol. 37:557–558.
 56. Dryden, M. S., S. Dally, and M. Crooch. 2004. A randomized, controlled trial of tea tree topical preparations versus a standard topical regimen for the clearance of MRSA colonization. J. Hosp. Infect. 56:283–288.
 57. Elliott, C. 1993. Tea tree oil poisoning. Med. J. Aust. 159:830–831.
 58. Elsom, G. K. F., and D. Hild. 1999. Susceptibility of methicillin-resistant *Staphylococcus aureus* to tea tree oil and mupirocin. J. Antimicrob. Chemother. 43:427–428.
 59. Ergin, A., and S. Arkan. 2002. Comparison of microdiffusion and disc diffusion methods in assessing the in vitro activity of fluconazole and *Melaleuca alternifolia* (tea tree) oil against vaginal *Candida* isolates. J. Chemother. 14:465–472.
 60. Felbati, H. M. 1960. Cajuput-type oil for the treatment of furunculosis. J. Natl. Med. Assoc. 52:25–34.
 61. Griffin, S. G., J. L. Markham, and D. N. Leach. 2000. An agar dilution method for the determination of the minimum inhibitory concentration of essential oils. J. Essent. Oil Res. 12:249–255.
 62. Griffin, S. G., S. G. Wyllie, and J. L. Markham. 1999. Determination of octanol-water partition coefficients for terpenoids using reversed-phase high-performance liquid chromatography. J. Chromatogr. A 864:221–228.
 63. Griffin, S. G., S. G. Wyllie, J. L. Markham, and D. N. Leach. 1999. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. Flav. Fragr. J. 14:322–332.
 64. Gruppo, F. C., J. C. Ramacciotti, R. P. Simoes, F. M. Florio, and A. Sartoratto. 2002. Antimicrobial activity of garlic, tea tree oil, and chlorhexidine against oral microorganisms. Int. Dent. J. 52:433–437.
 65. Gurether, E. 1988. Australian tea tree oils. Report of a field survey. Perfum. Essenc. Oil Res. 59:642–644.
 66. Gustafson, J. E., S. D. Cox, Y. C. Liew, S. G. Wyllie, and J. R. Warrington. 2001. The bacterial multi-antibiotic resistant (Mar) phenotype leads to increased tolerance to tea tree oil. Pathology 33:211–215.
 67. Gustafson, J. E., Y. C. Liew, S. G. Wyllie, J. Markham, H. C. Bell, S. G. Wyllie, and J. R. Warrington. 1998. Effects of tea tree oil on *Escherichia coli*. Lett. Appl. Microbiol. 26:194–198.
 68. Hada, T., S. Furuse, Y. Matsumoto, H. Hamashima, K. Masuda, K. Shigima, T. Arai, and M. Satsuta. 2001. Comparison of the effects in vitro of tea tree oil and plectonol on methicillin-susceptible and methicillin-resistant strains of *Staphylococcus aureus*. Microbiol. 106(Suppl. 2):133–141.
 69. Hada, T., Y. Inoue, A. Shirahashi, and H. Hamashima. 2003. Leakage of K⁺ ions from *Staphylococcus aureus* in response to tea tree oil. J. Microbiol. Methods 53:309–312.
 70. Halford, A. C. F. 1936. Diabetic gangrene. Med. J. Aust. 12:121–122.
 71. Hammer, K. A., C. F. Carson, and T. V. Riley. 2003. Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. J. Appl. Microbiol. 95:853–860.
 72. Hammer, K. A., C. F. Carson, and T. V. Riley. 2004. Antifungal effects of *Melaleuca alternifolia* (tea tree) oil and its components on *Candida albicans*, *Candida glabrata* and *Saccharomyces cerevisiae*. J. Antimicrob. Chemother. 53:1081–1085.
 73. Hammer, K. A., C. F. Carson, and T. V. Riley. 2000. In vitro activities of ketoconazole, econazole, miconazole, and *Melaleuca alternifolia* (tea tree) oil against *Malessezia* species. Antimicrob. Agents Chemother. 44:467–469.
 74. Hammer, K. A., C. F. Carson, and T. V. Riley. 2002. In vitro activity of *Melaleuca alternifolia* (tea tree) oil against dermatophytes and other filamentous fungi. J. Antimicrob. Chemother. 50:195–199.
 75. Hammer, K. A., C. F. Carson, and T. V. Riley. 1999. In vitro susceptibilities of *Aspergillus* and *Trichophyton* associated with tinea vaginosa to *Melaleuca alternifolia* (tea tree) oil. Antimicrob. Agents Chemother. 43:396.
 76. Hammer, K. A., C. F. Carson, and T. V. Riley. 1999. Influence of organic matter, cations and surfactants on the antimicrobial activity of *Melaleuca alternifolia* (tea tree) oil in vitro. J. Appl. Microbiol. 86:446–452.
 77. Hammer, K. A., C. F. Carson, and T. V. Riley. 1996. In-vitro activity of essential oils, in particular *Melaleuca alternifolia* (tea tree) oil and tea tree oil products, against *Candida* spp. J. Antimicrob. Chemother. 42:591–595.
 78. Hammer, K. A., C. F. Carson, and T. V. Riley. 2000. *Melaleuca alternifolia* (tea tree) oil inhibits germ tube formation by *Candida albicans*. Med. Mycol. 38:355–362.
 79. Hammer, K. A., C. F. Carson, and T. V. Riley. 1996. Susceptibility of transient and commensal skin flora to the essential oil of *Melaleuca alternifolia* (tea tree) oil. Am. J. Infect. Control 24:186–189.
 80. Hammer, K. A., L. Dry, M. Johnson, E. M. Michalak, C. F. Carson, and T. V. Riley. 2003. Susceptibility of oral bacteria to *Melaleuca alternifolia* (tea tree) oil in vitro. Oral Microbiol. Immunol. 18:389–392.
 81. Hart, P. H., C. Brand, C. F. Carson, T. V. Riley, R. H. Prager, and J. J. Finlay-Jones. 2000. Terpinen-4-ol, the major component of the essential oil of *Melaleuca alternifolia* (tea tree) oil, suppresses inflammatory mediator production by activated human monocytes. Inflamm. Res. 49:619–626.
 82. Hausen, B. M., J. Rehling, and M. Hinkelshausen. 1999. Degradation products of monoterpenes are the sensitizing agents in tea tree oil. Am. J. Contact Dermatitis 10:66–77.
 83. Hower, L. E., D. N. Leach, D. L. S. Lee, R. J. Henry, and P. R. Beyerle. 2000. Natural variation in the essential oil content of *Melaleuca alternifolia* (tea tree). Biochem. Syst. Ecol. 28:367–382.
 84. Humphrey, E. M. 1950. A new Australian germicide. Med. J. Aust. 1:417–418.
 85. Inouye, S., T. Takizawa, and H. Yamaguchi. 2001. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. J. Antimicrob. Chemother. 47:565–573.
 86. Inouye, S., T. Tsurukawa, M. Watanabe, K. Takeda, M. Akao, Y. Nishiyama, and H. Yamaguchi. 2000. Inhibitory effect of essential oils on apical growth of *Aspergillus fumigatus* by vapour contact. Mycoses 43:17–23.
 87. Inouye, S., K. Uchida, and H. Yamaguchi. 2001. In-vitro and in-vivo anti-*Trichophyton* activity of essential oils by vapour contact. Mycoses 44:99–107.
 88. Inouye, S., M. Watanabe, Y. Nishiyama, K. Takeda, M. Akao, and H. Yamaguchi. 1998. Antisporulating and respiration-inhibitory effects of essential oils on filamentous fungi. Mycoses 41:403–410.
 89. International Organization for Standardization. 2004. ISO 4730:2004. Oil of *Melaleuca*, terpinen-4-ol type (tea tree oil). International Organization for Standardization, Geneva, Switzerland.
 90. Jackson, R. W., and J. A. McMas. 1965. Effects of toluene on *Escherichia coli*. J. Bacteriol. 90:1420–1424.
 91. Jacobs, M. R., and C. S. Hornfield. 1994. *Melaleuca* oil poisoning. J. Toxicol. Clin. Toxicol. 32:461–464.
 92. Jandorek, A., K. A. Vaishampayan, and J. A. Vazquez. 1998. Efficacy of melaleuca oil solution for the treatment of fluconazole refractory oral candidiasis in AIDS patients. AIDS 12:1033–1037.
 93. Johns, M. R., J. E. Johns, and V. Rudolph. 1992. Steam distillation of tea tree (*Melaleuca alternifolia* oil). J. Sci. Food Agric. 58:49–53.
 94. Khalil, Z., A. L. Pearce, N. Sankaranarayanan, E. Storer, J. J. Finlay-Jones, and P. H. Hart. 2004. Regulation of wheal and flare by tea tree oil: complementary human and rodent studies. J. Invest. Dermatol. 123:683–690.
 95. Klineck, J. K., R. Nowicki, K. Sundell, M. Konishi, R. Roemer, G. Hanke, and W. Krol. 2002. Application of a tea tree oil and its preparations in combined treatment of dermatomycoses. Mikol. Lekarska 55:93–95.
 96. Knight, T. E., and B. M. Hausen. 1994. *Melaleuca* oil (tea tree oil) dermatitis. J. Am. Acad. Dermatol. 30:423–427.
 97. Koh, K. J., A. L. Pearce, G. Marshman, J. J. Finlay-Jones, and P. H. Hart. 2002. Tea tree oil reduces histamine-induced skin inflammation. Br. J. Dermatol. 147:1212–1217.
 98. Lassak, E. V., and T. McCarthy. 1963. Australian medicinal plants, p. 93–99, 115. Methuen Australia, North Ryde, Australia.
 99. Lungbottom, C. J., C. F. Carson, K. A. Hammer, B. J. Mee, and T. V. Riley. 2004. Tolerance of *Pseudomonas aeruginosa* to *Melaleuca alternifolia* (tea tree) oil is associated with the outer membrane and energy-dependent cellular processes. J. Antimicrob. Chemother. 54:386–392.
 100. Low, D., B. D. Rawal, and W. J. Griffin. 1974. Antibacterial activity of the essential oils of some Australian Myrtaceae with special reference to the activity of chromatographic fractions of oil of *Eucalyptus cinerifolia*. Planta Med. 26:184–189.
 101. Low, T. 1990. Bush medicine. Harper Collins Publishers, North Ryde, NSW, Australia.
 102. MacDonald, V. D. 2003. The rationale of treatment. Aust. J. Dent. 34:281–285.
 103. Mann, C. M., S. D. Cox, and J. L. Markham. 2000. The outer membrane of *Pseudomonas aeruginosa* NCTC 6749 contributes to its tolerance to the essential oil of *Melaleuca alternifolia* (tea tree) oil. Lett. Appl. Microbiol. 30:294–297.

104. Mann, C. M., and J. L. Markham. 1998. A new method for determining the minimum inhibitory concentration of essential oils. *J. Appl. Microbiol.* 84:538–544.
105. Maruzellu, J. C., and N. A. Siccardi. 1960. Antibacterial activity of essential oil vapors. *J. Am. Pharm. Assoc.* 49:692–694.
106. May, J., C. H. Chan, A. King, L. Williams, and G. L. French. 2000. Time-kill studies of tea tree oils on clinical isolates. *J. Antimicrob. Chemother.* 45:639–643.
107. Messenger, S., K. A. Hammer, C. F. Carson, and T. V. Riley. 2005. Assessment of the antibacterial activity of tea tree oil using the European EN 1276 and EN 12054 standard suspension tests. *J. Hosp. Infect.* 59:113–125.
108. Messenger, S., K. A. Hammer, C. F. Carson, and T. V. Riley. 2005. Effectiveness of hand-cleaning formulations containing tea tree oil assessed *ex vivo* on human skin and *in vivo* with volunteers using European standard EN 1499. *J. Hosp. Infect.* 59:220–228.
109. Mikus, J., M. Harkenthal, D. Stewarding, and J. Rethling. 2000. *In vitro* effect of essential oils and isolated mono- and sesquiterpenes on *Leishmania major* and *Trypanosoma brucei*. *Planta Med.* 66:366–368.
110. Minami, M., M. Kito, T. Nakaya, T. Yamamoto, H. Kurayama, and J. Imanishi. 2003. The inhibitory effect of essential oils on herpes simplex virus type-1 replication *in vitro*. *Microbiol. Immunol.* 47:681–694.
111. Mundello, F., F. De Bernardis, A. Girolamo, G. Salvatore, and A. Cassone. 2003. *In vitro* and *in vivo* activity of tea tree oil against azole-susceptible and -resistant human pathogenic yeasts. *J. Antimicrob. Chemother.* 51:1223–1229.
112. Morris, M. C., A. Donoghue, J. A. Mrkewitz, and K. C. Osterhout. 2003. Ingestion of tea tree oil (Melaleuca oil) by a 4-year-old boy. *Pediatr. Emerg. Care* 19:169–171.
113. Martag, J. G. 1999. Biomas and oil production of tea tree, p. 109–133. In J. Southwell and R. Love (ed.), *Tea tree: the genus Melaleuca*, vol. 9. Harwood Academic Publishers, Amsterdam, The Netherlands.
114. Nelson, R. R. S. 2000. Selection of resistance to the essential oil of *Melaleuca alternifolia* in *Staphylococcus aureus*. *J. Antimicrob. Chemother.* 45:549–550.
115. Nelson, R. R. S. 1997. *In vitro* activities of five plant essential oils against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium*. *J. Antimicrob. Chemother.* 40:305–306.
116. Nenoff, P., U.-F. Haustein, and W. Brandt. 1996. Antifungal activity of the essential oil of *Melaleuca alternifolia* (tea tree oil) against pathogenic fungi *in vitro*. *Skin Pharmacol.* 9:388–394.
117. Oliva, B., E. Piccinilli, T. Pedini, E. Pontieri, P. Aureli, and A. Ferrini. 2003. Antimicrobial activity of *Melaleuca alternifolia* essential oil and its major components. *Leti. Appl. Microbiol.* 37:185–187.
118. Opdyke, D. J. 1975. Fragrance raw materials monographs (eucalyptol). Food Cosmet. Toxicol. 13:105–106.
119. Penrot, A., J. J. Fluty-Jones, and P. H. Hart. 2005. Reduction of nickel-induced contact hypersensitivity reactions by topical tea tree oil in humans. *Inflamm. Res.* 54:22–30.
120. Peña, E. F. 1962. *Melaleuca alternifolia* oil—its use for trichomonas vaginitis and other vaginal infections. *Obstet. Gynecol.* 19:793–795.
121. Penfold, A. R., and R. Grant. 1925. The germicidal values of some Australian essential oils and their pure constituents, together with those for some essential oil isolates, and synthetics. Part III. *J. R. Soc. New South Wales* 59:346–349.
122. Penfold, A. R., and R. Grant. 1923. The germicidal values of the principal commercial *Eucalyptus* oils and their pure constituents, with observations on the value of concentrated disinfectants. *J. R. Soc. New South Wales* 57:80–89.
123. Penfold, A. R., and R. Grant. 1924. The germicidal values of the pure constituents of Australian essential oils, together with those for some essential oil isolates and synthetics. Part II. *J. R. Soc. New South Wales* 58:117–123.
124. Penfold, A. R., and F. R. Morrison. 1946. Bulletin no. 14. Australian tea tree of economic value, part 1, 3rd ed. Thomas Henry Tennant, Government Printer, Sydney, Australia.
125. Perry, N. B., N. J. Brennan, J. W. Van Klink, W. Harris, M. H. Douglas, J. A. Gimpey, B. M. Smallfield, and A. R. E. 1997. Essential oils from New Zealand manuka and kanuka: chemotaxonomy of *Leptospermum*. *Phytochemistry* 44:1485–1494.
126. Ranaiv, A., U. Weir, and S. F. Bloomfield. 1995. Antimicrobial effects of tea-tree oil and its major components on *Staphylococcus aureus*, *Staph. epidermidis* and *Propionibacterium acnes*. *Leti. Appl. Microbiol.* 21:242–245.
127. Rethling, J., A. Weseler, U. Lindvatter, and R. Salter. 2002. Bioactive essential oils used in phytonomic as antifungal agents: Australian tea tree oil and manuka oil. *Acta Phytotherapeutica* 1:06–32.
128. Ruston, R. T., N. W. Davis, J. C. Pags, and C. A. Durkin. 1997. The effect of tea tree oil extract on the growth of fungi. *Lower Extremity* 4:113–116.
129. Russell, M. 1999. Toxicology of tea tree oil, p. 191–201. In J. Southwell and R. Lowe (ed.), *Tea tree: the genus Melaleuca*, vol. 9. Harwood Academic Publishers, Amsterdam, The Netherlands.
130. Satchell, A. C., A. Saurajen, C. Bell, and R. S. Barnetson. 2002. Treatment of dandruff with 5% tea tree oil shampoo. *J. Am. Acad. Dermatol.* 47:852–855.
131. Satchell, A. C., A. Saurajen, C. Bell, and R. S. Barnetson. 2002. Treatment of interdigital tinea pedis with 25% and 50% tea tree oil solution: a randomized, placebo controlled, blinded study. *Australas. J. Dermatol.* 43:175–178.
132. Schatzler, P., K. Schön, and J. Rethling. 2001. Antiviral activity of Australian tea tree oil and eucalyptus oil against herpes simplex virus in cell culture. *Pharmazie* 56:343–347.
133. Seawright, A. 1993. Tea tree oil poisoning. *Med. J. Aust.* 159:831.
134. Shapiro, S., A. Meier, and B. Guggenheim. 1994. The antimicrobial activity of essential oils and essential oil components towards oral bacteria. *Oral Microbiol. Immunol.* 9:202–208.
135. Shemko, A., and W. L. Mayo. 1991. Australian tea tree oil: a natural antiseptic and fungicidal agent. *Aust. J. Pharm.* 72:802–803.
136. Sherry, E., H. Boeck, and P. H. Warnke. 2001. Topical application of a new formulation of eucalyptus oil phytochemicals elicits methicillin-resistant *Staphylococcus aureus* infection. *Am. J. Infect. Control* 29:346.
137. Shin, S. 2003. Anti-*Aspergillus* activities of plant essential oils and their combination effects with ketoconazole or amphotericin B. *Arch. Pharmacol. Res.* 26:389–393.
138. Sikkema, J. J., A. M. de Bont, and B. Poolman. 1995. Mechanisms of membrane toxicity of hydrocarbons. *Microbiol. Rev.* 59:201–222.
139. Soukoulis, S., and R. Hirsch. 2004. The effects of a tea tree oil-containing gel on plaque and chronic gingivitis. *Ant. Dent. J.* 49:78–83.
140. Southwell, L., A. J. Hayes, J. Markham, and D. N. Lench. 1993. The search for optimally bioactive Australian tea tree oil. *Acta Hort.* 344:236–265.
141. Southwell, L. A., S. Freeman, and D. Rubel. 1997. Skin irritation of tea tree oil. *J. Essent. Oil Res.* 9:47–52.
142. Swords, G., and G. L. K. Hunter. 1978. Composition of Australian tea tree oil (*Melaleuca alternifolia*). *J. Agric. Food Chem.* 26:734–737.
143. Syed, T. A., Z. A. Qureshi, S. M. Ali, S. Ahmad, and S. A. Ahmad. 1999. Treatment of topical onychomycosis with 2% lufenalene and 5% *Melaleuca alternifolia* (tea tree) oil in cream. *Trop. Med. Int. Health* 4:284–287.
144. Takaranda, K. 2005. The effects of essential oils on periodontopathogenic bacteria and oral halitosis. *Oral Dis.* 11:115.
145. Tong, M., M. P. Altman, and R. S. Barnetson. 1992. Tea tree oil in the treatment of tinea pedis. *Ant. J. Dermatol.* 33:145–149.
146. Uribe, S., J. Ramirez, and A. Peña. 1985. Effects of β -pinene on yeast membrane functions. *J. Bacteriol.* 161:1195–1200.
147. Uribe, S., P. Rangel, G. Espinoza, and G. Aguirre. 1990. Effects of cyclohexane, an industrial solvent, on the yeast *Saccharomyces cerevisiae* and on isolated yeast mitochondria. *Appl. Environ. Microbiol.* 56:2114–2119.
148. van der Valk, P., G. A. C. de Groot, D. P. Bruyzeel, P. J. Coenraads, and J. W. Weijland. 1994. Allergic contact eczema due to 'tea tree' oil. *Ned. Tijdschr. Geneesk.* 138:823–825.
149. Vazquez, J. A., M. T. Arganzon, D. Bolivar, J. K. Valsarapany, and R. A. Atkins. 2000. *In vitro* susceptibilities of *Candida* and *Aspergillus* species to *Melaleuca alternifolia* (tea tree) oil. *Rev. Iberoam. Micol.* 17:60–63.
150. Vazquez, J. A., and A. A. Zawawi. 2002. Efficacy of alcohol-based and alcohol-free melaleuca oral solution for the treatment of fluconazole-refractory oropharyngeal candidiasis in patients with AIDS. *HIV Clin. Trials* 3:379–385.
151. Veien, N. K., K. Rosner, and G. Skovgaard. 2004. Is tea tree oil an important contact allergen? *Contact Dermatitis* 50:378–379.
152. Vulliam, D., D. Mandin, and J. P. Chaudmont. 1996. Activités antagonistes, *in vitro*, de quelques huiles essentielles et de composés naturels volatils vis à vis de la croissance de *Trichomonas vaginalis*. *Phytotherapie* 67:279–281.
153. Walther, M. 1972. Clinical investigation of Australian *Melaleuca alternifolia* oil for a variety of common foot problems. *Curr. Podiatry* 19:72–73.
154. Walsh, L. J., and J. Longstaff. 1987. The antimicrobial effects of an essential oil on selected oral pathogens. *Periodontology* 8:11–15.
155. Warnke, P. H., E. Sherry, P. A. Russo, M. Sprengel, V. Acil, J. P. Brede, S. Schubert, J. Willfang, and I. Springer. 2005. Antibacterial clinical oils reduce tumor skin inflammation in cancer patients. *J. Clin. Oncol.* 23:1588–1589.
156. Weiss, E. A. 1997. Essential oil crops. CAB International, New York, N.Y.
157. Williams, L. R., and V. N. Hume. 1983. Plantation production of oil of melaleuca (tea tree oil)—a re-evaluated Australian essential oil industry. *Search* 19:294–297.
158. Williams, L. R., V. N. Hume, and S. Acre. 1990. Antimicrobial activity of oil of melaleuca (tea tree oil). Its potential use in cosmetics and toiletries. *Cosmet. Aerosols Toiletries Aust.* 4:12–13, 16–18, 22.
159. Williams, L. R., V. N. Hume, and I. Lusuzi. 1993. An evaluation of the contribution of cineole and terpinen-4-ol to the overall antimicrobial activity of tea tree oil. *Cosmet. Aerosols Toiletries Aust.* 7:23–34.